A TRIDENT SCHOLAR PROJECT REPORT

NO. 161

"Receptor-Carrier Based Models for Antigen-Antibodv Interactions"

UNITED STATES NAVAL ACADEMY
ANNAPOLIS, MARYLAND

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER U.S.N.A. - TSPR; no. 161 (1989)	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* RECEPTOR-CARRIER BASED MODEL FOR ANTIGEN-ANTIBODY INTERACTIONS.		5. TYPE OF REPORT & PERIOD COVERED Final 1988/89
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Joel D. Stewart		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS United States Naval Academy, Annapolis.		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS United States Naval Academy, Annapolis.		12. REPORT DATE 7 July 1989
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)*
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

This document has been approved for public release; its distribution is UNLIMITED.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Accepted by the U.S. Trident Scholar Committee.

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Biological models
Antigen-Antibody reactions
Immunology

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

A theoretical model was developed for antigen-antibody interaction in a receptor-carrier based system. Many models have been developed, both kinetic and equilibrium, for receptor-to-ligand interaction in the immune response. The immune response is extremely complex, with very large numbers of receptor sites on many distinct cell types in the system. (OVER)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Therefore the system needs to have many restrictions upon it to make it manageable. Most models assume the area for interaction is an infinite sheet (i.e. an endless two dimensional cell membrane). These models are limited to only discussing the average number and types of interactions. This model is receptor-carrier based which counts interaction on a cell/vesicle surface to allow questions about actions and conditions per cell to be addressed. The models which have been addressed are the "sandwich" model which takes a two-step interaction with a linking ligand and then the antibody, and the agglutination model which addresses the inter-cellular binding between two adjacent cells through a bridging ligand. Results show that the sandwich model and the agglutination model are less sensitive immunoassay techniques than the direct binding assay, although these models are very useful for describing more complex interactions.

U.S.N.A. - Tident Scholar project report; no. 161 (1989)

"Receptor-Carrier Based Models for Antigen-Antibody Interactions"

A Trident Scholar Project Report

by

Midshipman First Class Joel D. Stewart, 1989

U. S. Naval Academy

Annapolis, Maryland

Boyd A. Waite

Assoc. Prof. Boyd A. Waite - Chemistry

Accepted for Trident Scholar Committee

Dennis F. Hasson

Chairperson

7 July 1989

Date

ABSTRACT

A theoretical model was developed for antigen-antibody interaction in a receptor-carrier based system. Many models have been developed, both kinetic and equilibrium, for receptor-to-ligand interaction in the immune response. The immune response is extremely complex, with very large numbers of receptor sites on many distinct cell types in the system. Therefore the system needs to have many restrictions upon it to make it manageable. Most models assume the area for interaction is an infinite sheet (i.e. an endless two dimensional cell membrane). These models are limited to only discussing the average number and types of interactions. This model is receptor-carrier based which counts interactions on a cell/vesicle surface to allow questions about actions and conditions per cell to be addressed. The models which have been addressed are the "sandwich" model which takes a two-step interaction with a linking ligand and then the antibody, and the agglutination model which addresses the inter-cellular binding between two adjacent cells through a bridging ligand. Results show that the sandwich model and the agglutination model are less sensitive immunoassay techniques than the direct binding assay, although these models are very useful for describing more complex interactions.

TABLE OF CONTENTS

I. INTRODUCTION

The immune response in humans is a highly organized and very specific defense system [1]. The immune system defends the body against foreign molecules, cells, or viruses, which are called antigens. The human system not only recognizes non-human cells, but also those from other individuals, which explains the difficulty in transplanting organs from one person to another [2].

There are two main types of cells, lymphocytes, which are involved in the immune response: T-cells (which mature in the thymus) and B-cells (which mature in bone marrow). The humoral immune response is produced by the B-cells and is most effective against pathogenic (disease causing) cells, viruses and free toxins. The cell-mediated immune response uses the T-cells and is effective against larger cells, such as parasites, cancer, and infected cells.

The cell-mediated response (Figure 1) is more complex than the humoral immune response (Figure 2). This is the system which is attacked by the AIDS virus. In many responses the antigen is picked up by a macrophage and is presented to a "virgin" T-cell which initiates a response. The simplest T-cell response would then produce the cytotoxic T-cell which kills the antigen bearing cell and then looks for other antigens. The

Cell-Mediated Immune Response

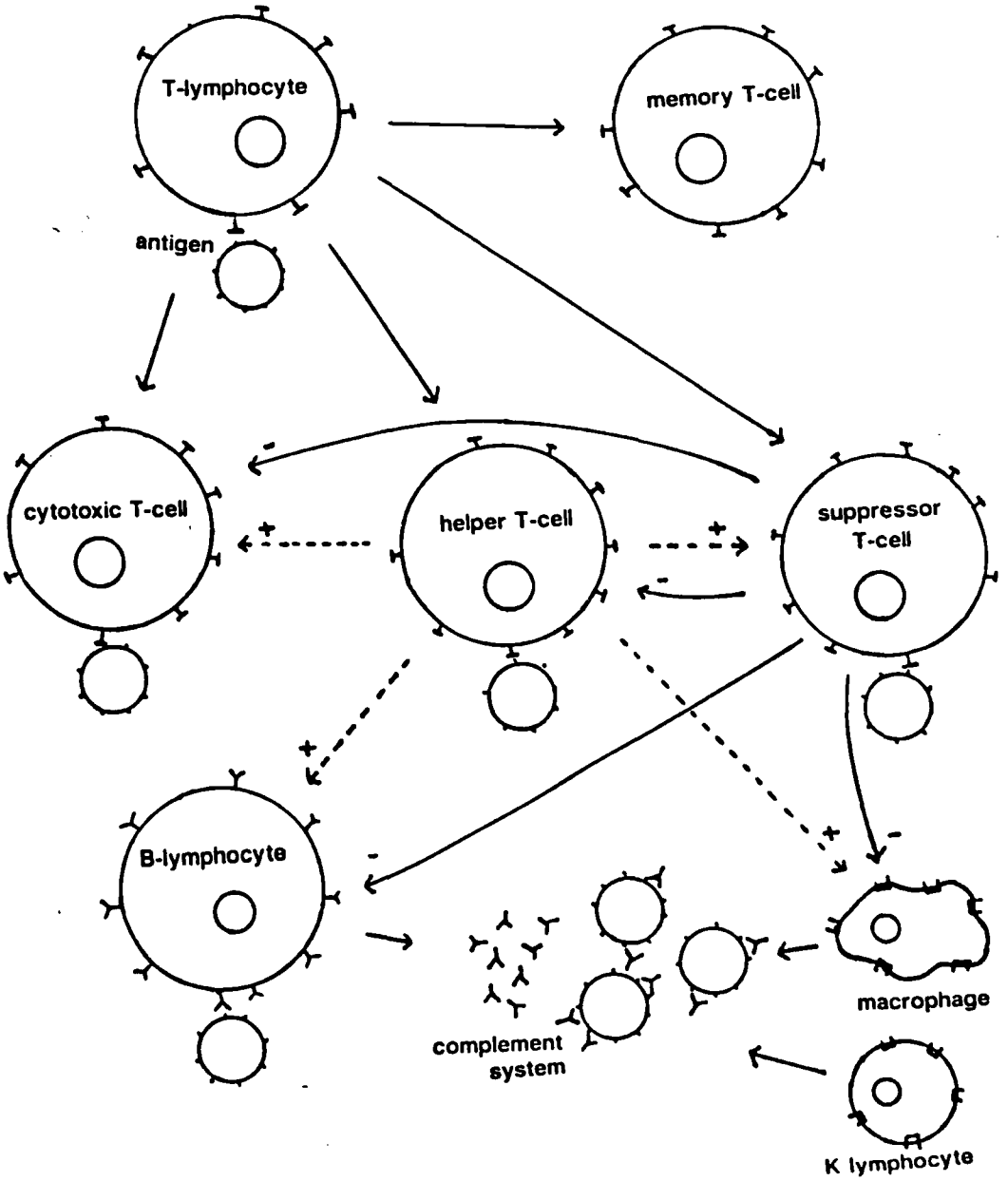


Figure 1

Humoral Immune Response

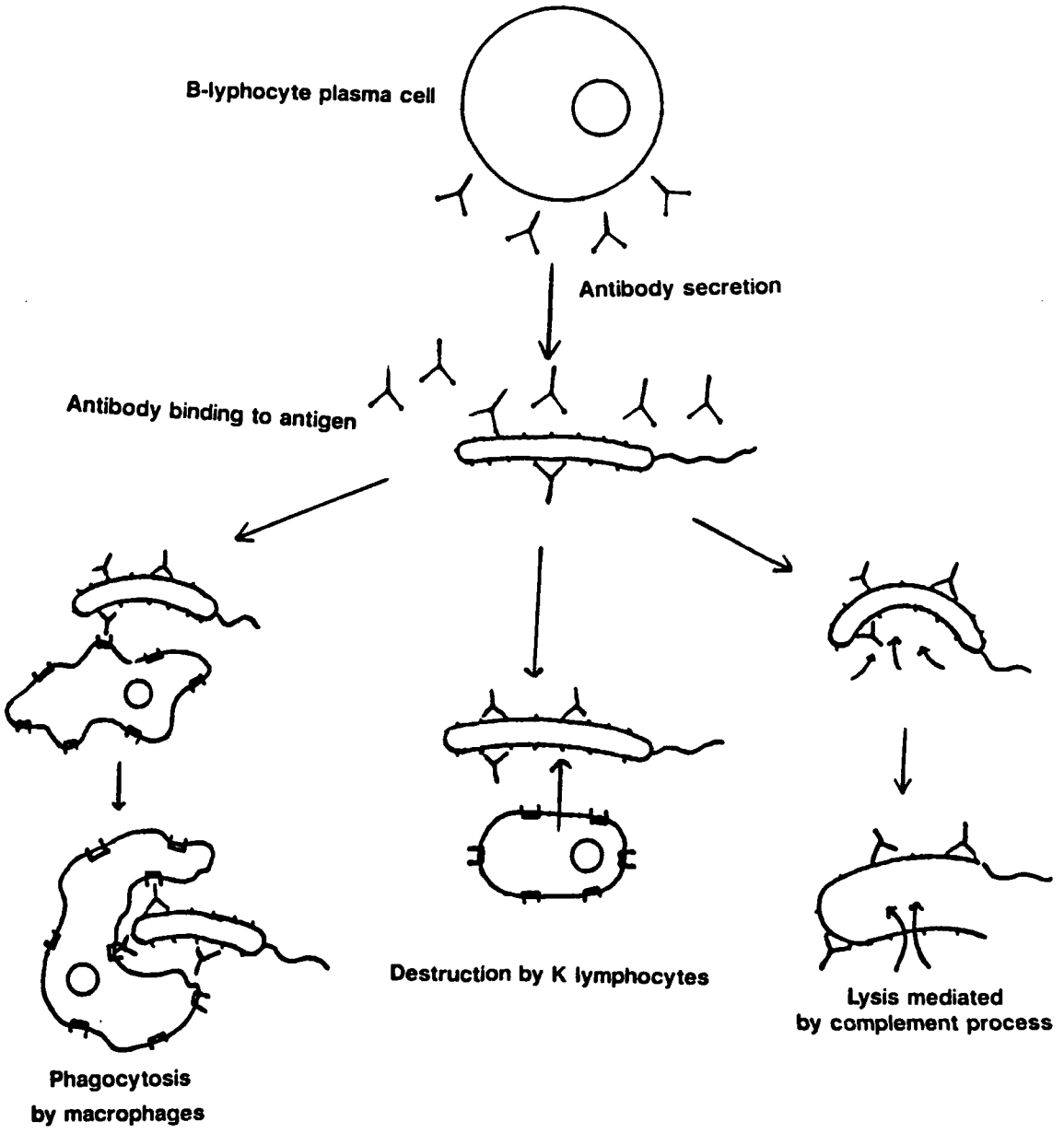


Figure 2

other response initiated by the T-cell involves helper T-cells and suppressor T-cells. The helper cells secrete lymphokines and interleukins which activate macrophages and mast cells in the area to ingest the antigen. The helper cells can also induce local plasma cells to produce antibodies. The helper cells also tell the antigen bound T-cells to reproduce. At the same time suppressor T-cells are doing the opposite of the helper cells in a dynamic balance to induce an appropriate level of response. An appropriate response is necessary since too little response will not rid the body of the antigen and over response is what causes side effects like sneezing, coughing and hives.

The B-cell response is more direct and simpler to understand. The B-cells move freely through the blood and lymph system of the body. When a B-cell encounters a specific antigen, it will begin to grow, and divide. This division produces two types of cells, the memory cells and the plasma cells. The memory cells allow a more rapid response to be initiated if the same antigen were to attack again. The plasma cells actually produce antibodies which are used to attack the antigen. Figure 3 shows the layout of a typical IgG antibody. There are more kinds of antibodies than just IgG, but IgG is the most common, with serum levels up to 12.0 mg/ml. The antibodies are made up of two light and two heavy protein

Antibody Structure

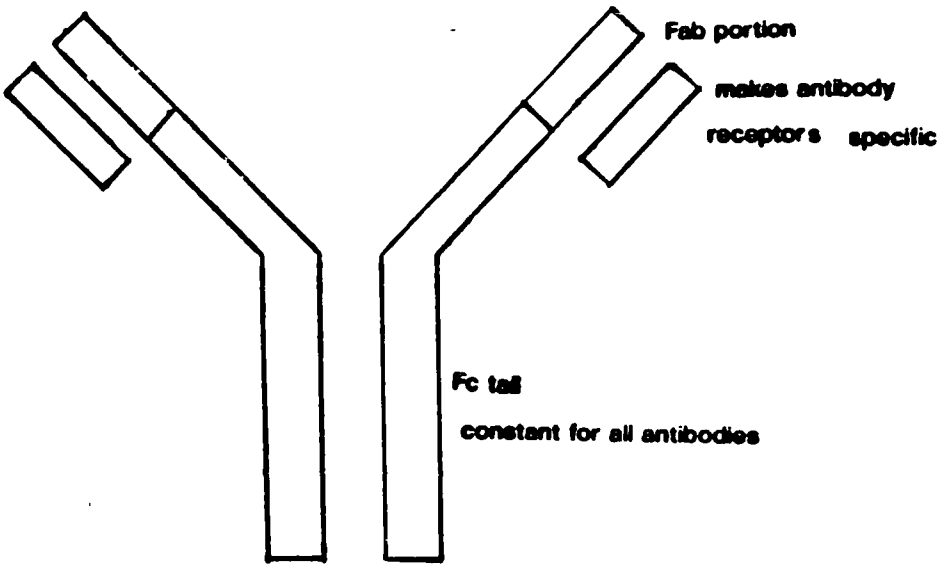


Figure 3

chains. Antibodies are very specific in their binding sites (Fab portion), and hence differ in the amino acid sequencing in that region, but all contain an Fc tail which is constant in amino acid sequencing from one antibody molecule to another. This Fc tail is the portion of the antibody which helps initiate the complement system. This system is shown in Figure 4. The chain of proteins forms a pathway to destroy the antigen, and acts as a catalyst to promote larger response further along in the process. The system has two methods of ridding the body of the antigen: macrophages in the area can be activated to engulf the antigen or to form a hole in the cell membrane of the antigen to lyse the cell. The humoral response can also produce K lyphocytes which mark the antigen and kill it through an unknown mechanism [2].

The purpose of this paper is to describe in detail the derivation of a theoretical model for cellular interaction. This interaction occurs in many places in the immune response; for example: antibody to antigen, macrophage to antigen, and helper T-cell to plasma cell.

Actual modeling of the B-cell response, considering the huge number of receptor sites on the B-cell [3], on an antigenic bacteria, or on a virus is unreasonable. The system needs to be easier to control and have fewer parameters. A more tractable system would be to model an

Complement System

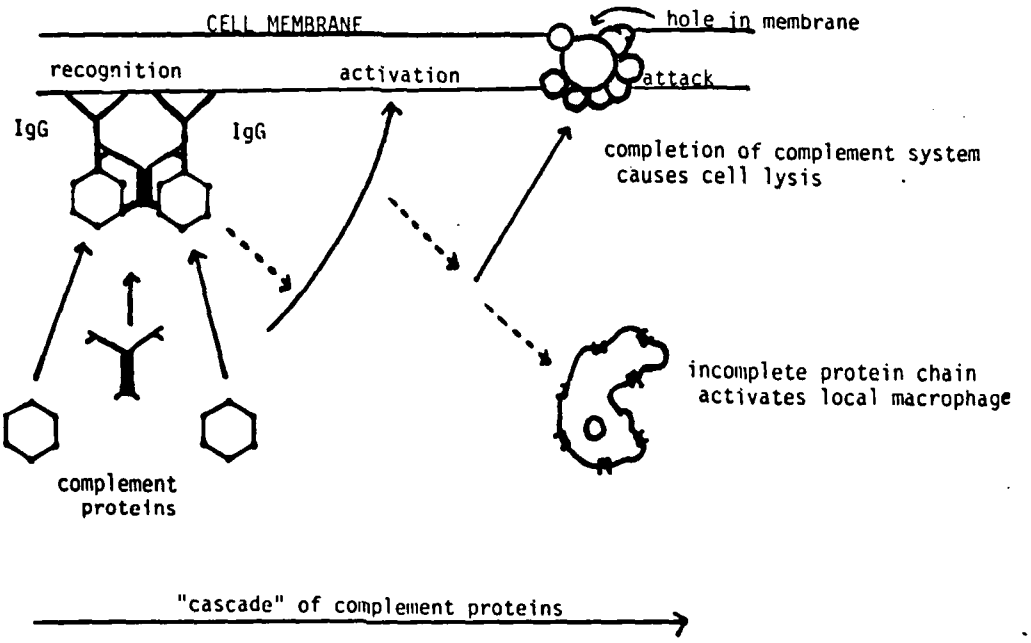


Figure 4

artificial system. The purpose of this paper is to describe in detail the derivation of a theoretical model for interactions within the immune system, its usefulness, and the results of two separate applications of the theoretical model.

The system which is being modeled uses vesicles in immunolysis assays, shown in Figure 5 [4]. These assays use covalently bound antigens on the surface of phospholipid vesicles [5]. The vesicles can be made with specific types and numbers of receptor sites. The vesicles can encapsulate markers like spin-labelled compounds [6] or fluorescent compounds like carboxyfluorescein [7]. Carboxyfluorescein does not fluoresce in high concentrations like that which is encapsulated in the vesicles, but when the vesicle is lysed through the complement process or by another means, the material is diluted and fluoresces. This system can be modeled and a series of questions can be answered such as the number of antigenic sites required to activate a response, and a theoretical limit to detection of antigen or antibody with this system.

Another more modern and exciting application of such modeling is for the system recently developed involving immunoadhesins for AIDS therapy (Figure 6) [8]. It has been shown that the AIDS virus kills by depleting the body's T4 lymphocytes (a type of T-cell) and the victim

Immunolysis Assays

monovalent or bivalent ligands or receptors

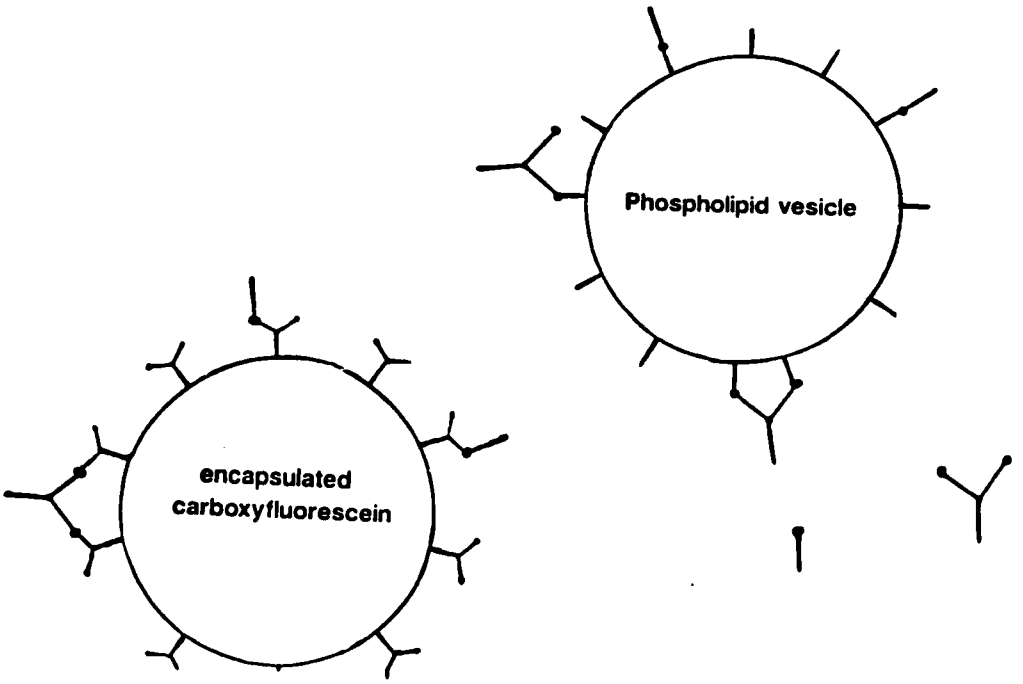


Figure 5

AIDS Virus Immunoadhesins

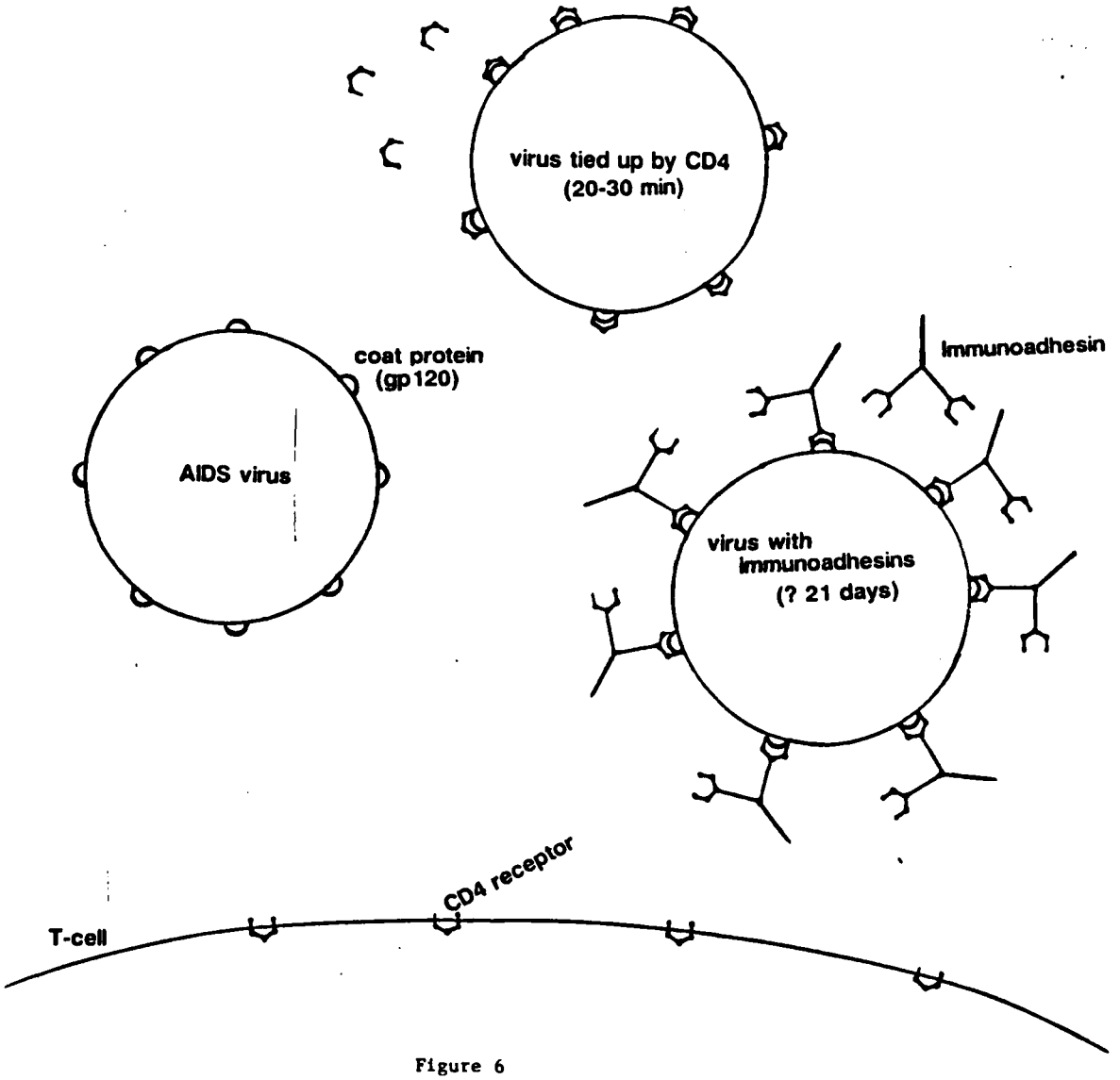


Figure 6

becomes susceptible to infections and neoplasms (new growth, tumors). The T4 lymphocytes have numerous CD4 protein sites as receptors. The Human Immunodeficiency Virus (HIV) envelope glycoprotein (gp120) has been shown to be strongly attracted to the CD4 protein. Most therapies are ineffective against the AIDS virus because its variable receptors keep changing and modifying. The one common denominator is its attraction to the CD4 site on the T-cells. After the virus has attached itself to the T-cell membrane, it is internalized, uncoated, and alters the cellular DNA. It would seem that an increase in free CD4 protein in the blood would tie up the coat protein on the virus (gp120) and not allow it to attach to the T-cells. The problem with this approach is that CD4 only has an effective lifespan of twenty to thirty minutes in the blood. Researchers have found a way to develop a special antibody, called an immunoadhesin, which has CD4 receptor sites and an Fc tail. Experiments predict a lifespan approaching 21 days, the same as for a natural IgG. The goal of this program is to tie up the virus so it cannot attack the T4 cells and allow the normal immune response, involving the Fc portion of the immunoadhesin and the complement system, to kill the virus. This system can be represented by monovalent receptors (the gp120 coat protein sites) on the viral surface and bivalent ligands, the immunoadhesins. This

model can describe the specific actions on the viral surface.

Many questions still remain about the specific methods and limiting characteristics of the immune system. A theoretical model would be useful to answer some of these questions. Since the antibody to antigen binding constant is ambiguous [9] and a crucial factor in explaining cell to cell interactions, it is a central goal of any theoretical model to find a realistic value for this constant. Varying this parameter while holding the other conditions to those of an actual experiment should allow this constant to be approximated. Attachment to the cell by first one protein followed by another is a possibility; modeling this two step process could show interesting effects. It is also believed that agglutination of the antigens into clumps helps the killer cells, macrophages, and the complement system to work more efficiently [1]. A model of these situations would help find the system's limits, characteristic constants, ideal conditions and number of bound antibodies needed to produce cell lysis.

The models in this paper are receptor-carrier based [10]. This form of modeling is significant since it allows questions to be asked concerning the number of antibodies bound or cross-links per cell/vesicle. Other models have been proposed [11,12] which are kinetic or

equilibrium studies of monovalent or multivalent receptors or ligands. These models assume the surface of interaction to be infinite so only questions of types or average number of structures can be asked. The counting done by this model keeps track of structures on the vesicle surface. It is assumed that the initial antibody attachment to the vesicles is caused by a receptor-ligand interaction. This initial attachment rate is considered to be independent of number of sites occupied if there are sites remaining (steric considerations are not taken into account). The subsequent attachment of multivalent antibodies (in the agglutination model, the bivalent receptor model, and the bivalent ligand sandwich model) do take steric effects into account. Finally it is assumed that the receptor sites are able to migrate freely [13] to allow adequate mixing for the equilibrium equations to be relevant.

II. GENERIC MODEL DERIVATION

The models are defined by a system of conservation equations. The derivations are not intuitive, so an example will be provided. The simplest case is one of monovalent receptors on the cell and monovalent ligands [14]. This system is represented in Figure 7.

This system has a vesicle, or cell, covered by n monovalent antigenic receptors. The binding ligands are monovalent, like Fab fragments. The system has a degeneracy, (i.e., number of arrangements) which is defined as d_i. Degeneracy is the number of equivalent arrangements of bound ligands.

$$d_i = n!/[i!(n-i)!] = \begin{pmatrix} n \\ i \end{pmatrix} \qquad (1)$$

This is derived by taking the number of possible arrangements of receptors (n!) and dividing by the number of arrangements of the different sites which are on the cell, including (i!) the number of identical sites which are bound and (n-i)! unbound sites. The ligands need to be specific for the receptor sites and are governed by the binding and dissociative rate constants, k_1 and k_{-1}. These constants are related to K, the equilibrium binding constant, by

$$K = k_1/k_{-1} \qquad (2)$$

Monovalent Receptor-Monovalent Ligand Model

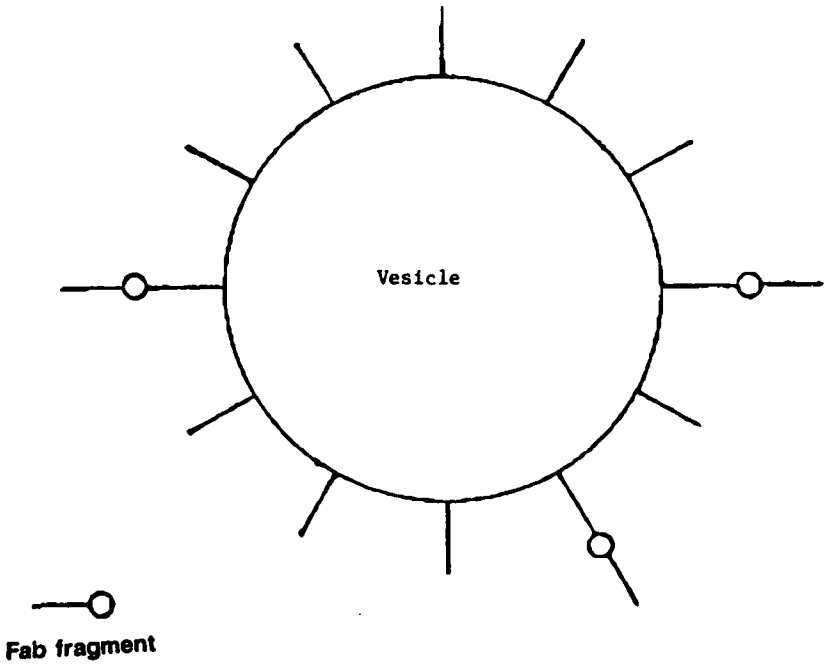


Figure 7

The Law of Mass Action representing the i th antibody binding is represented by

$$V_{i-1}' + a \overset{K}{=} V_i' \qquad (3)$$

which can be represented by the following arrangement where the ' represents one of the specific arrangements among the total d_i' possible arrangements of V_i, and "a" represents the free antibody concentration:

$$V_i' = (aK)V_{i-1}' \qquad (4)$$

The total concentration of V_i can be defined as

$$V_i' = V_i/d_i \qquad (5)$$

Repeating equation (4) i times gives the general equation

$$V_i = d_i(aK)^i V_o \qquad (6)$$

where V_o is the concentration of cells without any antibodies bound.

The total number of vesicles and antibodies in the system cannot change. This allows conservation equations to be found. The conservation equations for the

system can be derived as

$$V^o = \sum_{i=0}^{n} d_i (aK)^i V_o \qquad (7)$$

$$Ab^o = \sum_{i=0}^{n} i d_i (aK)^i V_o \qquad (8)$$

where V^o is the initial concentration of vesicles and Ab^o is the initial concentration of antibodies.

The mathematical identity of

$$\sum_{i=0}^{n} \binom{n}{i} x^i = (1+x)^n \qquad (9)$$

allows equation 7 to be reduced to

$$V^o = V_o(1+aK)^n \qquad (10)$$

and the derivative of equation 9 allows equation 8 to be reduced to

$$Ab^o = a + nV^o aK/(1+aK) \qquad (11)$$

A. General Derivation Summary

This is the format which will be used to derive all future models:

1. Identify possible structures and parameters governing receptor to ligand binding.

2. Calculate degeneracy through possible arrangements, including unbound sites.

3. Strip (stepwise) possible arrangements to V_o (entirely empty) arrangement.

4. Derive conservation equations for vesicles and antibodies.

5. Address specific questions.

III. SANDWICH MODEL

The first model which will be discussed in depth is the "sandwich" model [4]. This model is shown in Figure 8. This model is different from the monovalent receptor/ monovalent ligand model by the ligand being bivalent, with one end specific to the receptors on the vesicle, and the other specific to the monvalent antibody. Now the vesicle receptors can have three possible arrangements: empty, with the bivalent "link" attached (f), and having the link attached to an antibody and the receptor site (s).

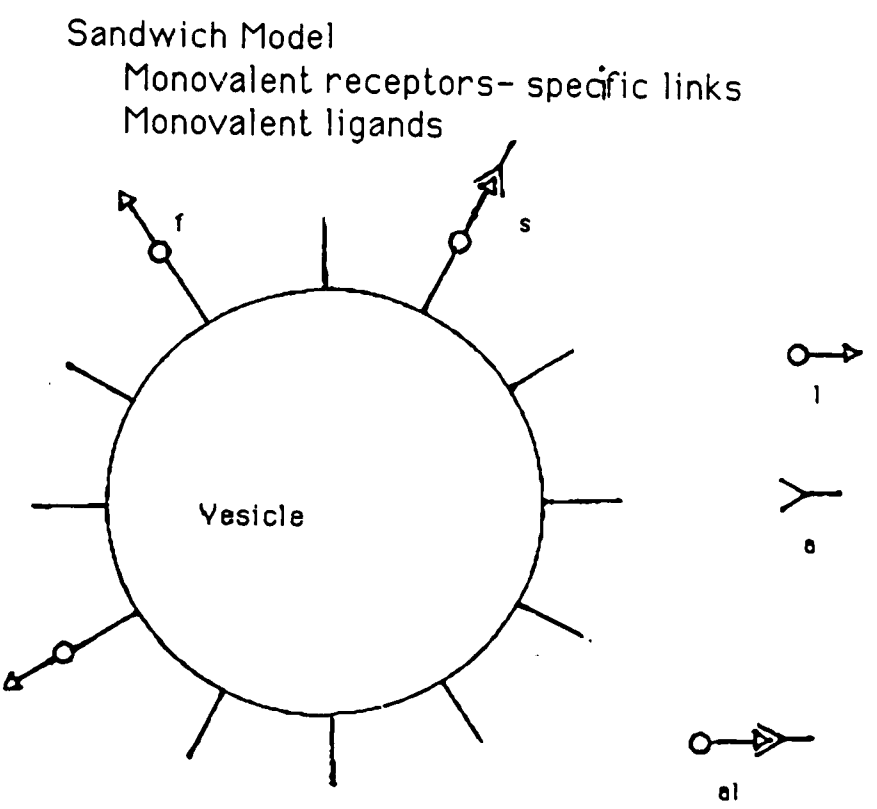

Sandwich Model
Monovalent receptors- specific links
Monovalent ligands
f
s
Vesicle
l
a
al

Figure 8

The degeneracy for this model is derived as in the monovalent model and it results in

$$d_{f,s} = n!/[f!s!(n-f-s)!] \qquad (12)$$

The Law of Mass Action for the model is represented by

$$V_{f,s}' \overset{K}{=} V_{f+1,s-1}' \qquad (13)$$

where K is represented by Equation 2 for the s(second) step. By repeating this equation s times the general equation for the concentration with f links and 0 sandwiches can be shown to be

$$V_{f,s}/d_{f,s} = (K_s a)^s V_{f+s,o}/d_{f+s,o} \qquad (14)$$

where a is the free antibody concentration and K_s is the equilibrium constant for that reaction. The Law of Mass Action equation

$$V_{f+s,o}/d_{f+s,o} = (K_f \ell)(K_s a)^s V_{f+s-1,o}/d_{f+s-1,o} \qquad (15)$$

can be repeated f+s times to yield the general equation

$$V_{f,s} = d_{f,s}(K_f \ell)^{f+s}(K_s a)^s V_{o,o} \qquad (16)$$

where ℓ is the free link concentration, and $V_{o,o}$ is the concentration of vesicles without any links or sandwiches attached.

The conservation equations can be derived to yield

$$V^o = \sum_{f=0}^{n} \sum_{s=0}^{n-f} d_{f,s}(K_f\ell)^{f+s}(K_s a)^s V_{o,o} \qquad (17)$$

$$Ab^o = a + K_s a l + \sum_{f=0}^{n} \sum_{s=0}^{n-f} (s) d_{f,s}(K_f\ell)^{f+s}(K_s a)^s V_{o,o} \qquad (18)$$

$$L^o = \ell + K_s a \ell + \sum_{f=0}^{n} \sum_{s=0}^{n-f} (f+s) d_{f,s}(K_f\ell)^{f+s}(K_s a)^s V_{o,o} \qquad (19)$$

which can be simplified with Equation 9 and its derivative to the formulas below

$$V^o = (1+xy+y)^n V_{o,o} \qquad (20)$$

$$Ab^o = a + K_s a\ell + nxy(1+xy)^{n-1}(1+y+xy)^{n-1} (1+y+xy-y/(1+xy))V_{o,o} \qquad (21)$$

$$L^o = \ell + K_s a\ell + ny(1+xy)^{n-1}(1+y+xy)^{n-1} [1+x(1+2y+xy-y/(1+xy)]V_{o,o} \qquad (22)$$

where $x = K^{s^a}$

$y = K_f\ell$

A. Sandwich Model Results

The sandwich model is best used to represent possible applications in immunolysis assays [4]. A vesicle can be made with antibody receptors, antigenic links, and antibody ligands. This would provide more flexibility in the types of systems to be modelled. In Figures 9 and 10 the fraction of vesicles with at least one receptor/link/ligand sandwich is shown with typical values for the binding constants for both binding steps, K_f and K_s. The value of 8.3×10^{-9} M for the constants L^o and Ab^o was chosen to allow for comparison with previous models. In Waite and Chang's paper on the monvalent receptor and monovalent ligand [14], results were shown for varying values of n (number of receptor sites) versus fraction of vesicles with one or more antibodies bound to the antigenic receptors (Figure 11). At values of n = 100 and $Ab^o = 8.3 \times 10^{-9}$ M, approximately seven percent of the vesicles had one or more ligands bound. In the sandwich model, with the link concentration held at the value of 8.3×10^{-9} M it takes relatively large concentrations of Ab^o, about 8.8×10^{-6} M, to reach the same seven percent. The sandwich model also allows the antibody concentration to be held constant and the link concentration to be varied. In this context the link concentration would need to be about 7.9×10^{-5} M, with the $[Ab^o] = 8.3 \times 10^{-9}$ M, to

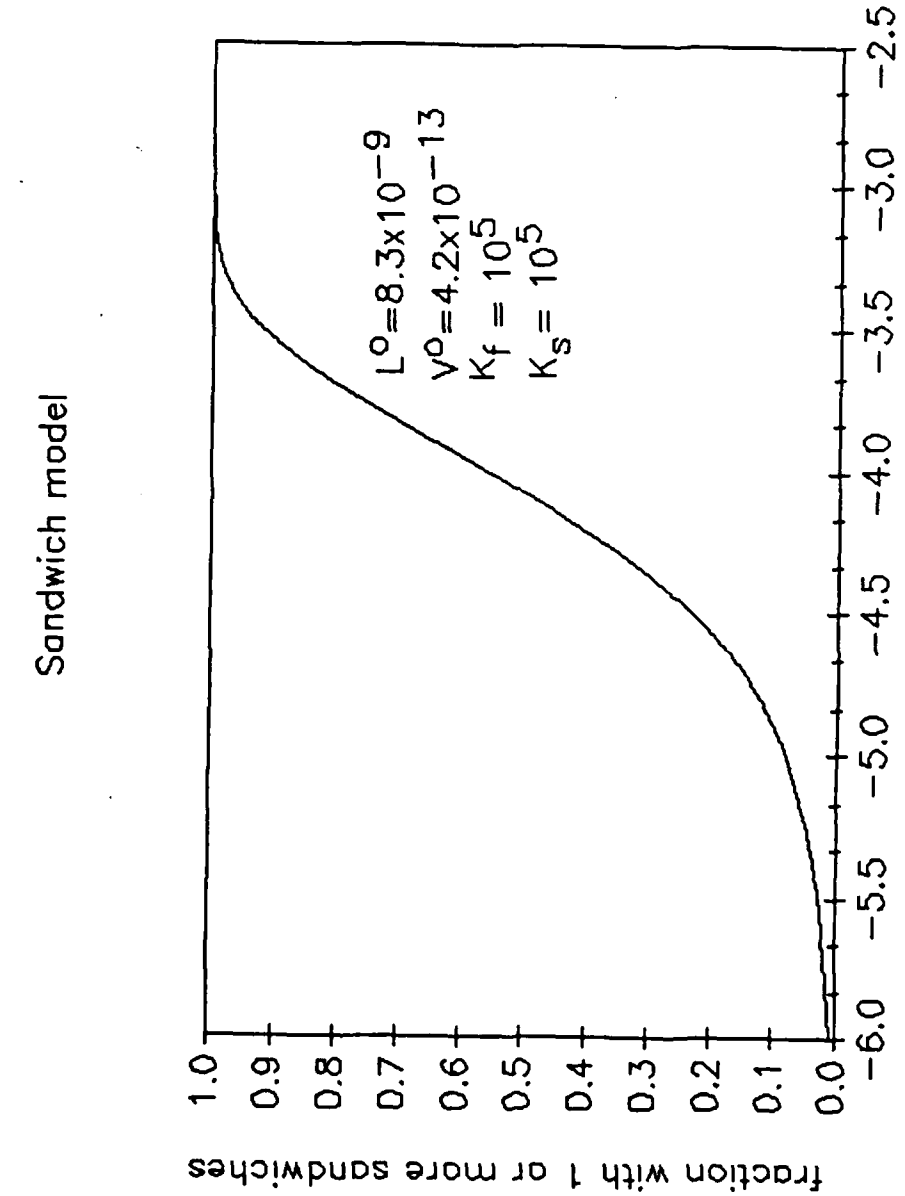

Sandwich model
$L^o=8.3x10^{-9}$
$V^o=4.2x10^{-13}$
$K_f = 10^5$
$K_s = 10^5$
fraction with 1 or more sandwiches
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-6.0
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
-2.5
Log Ab^o

Figure 9

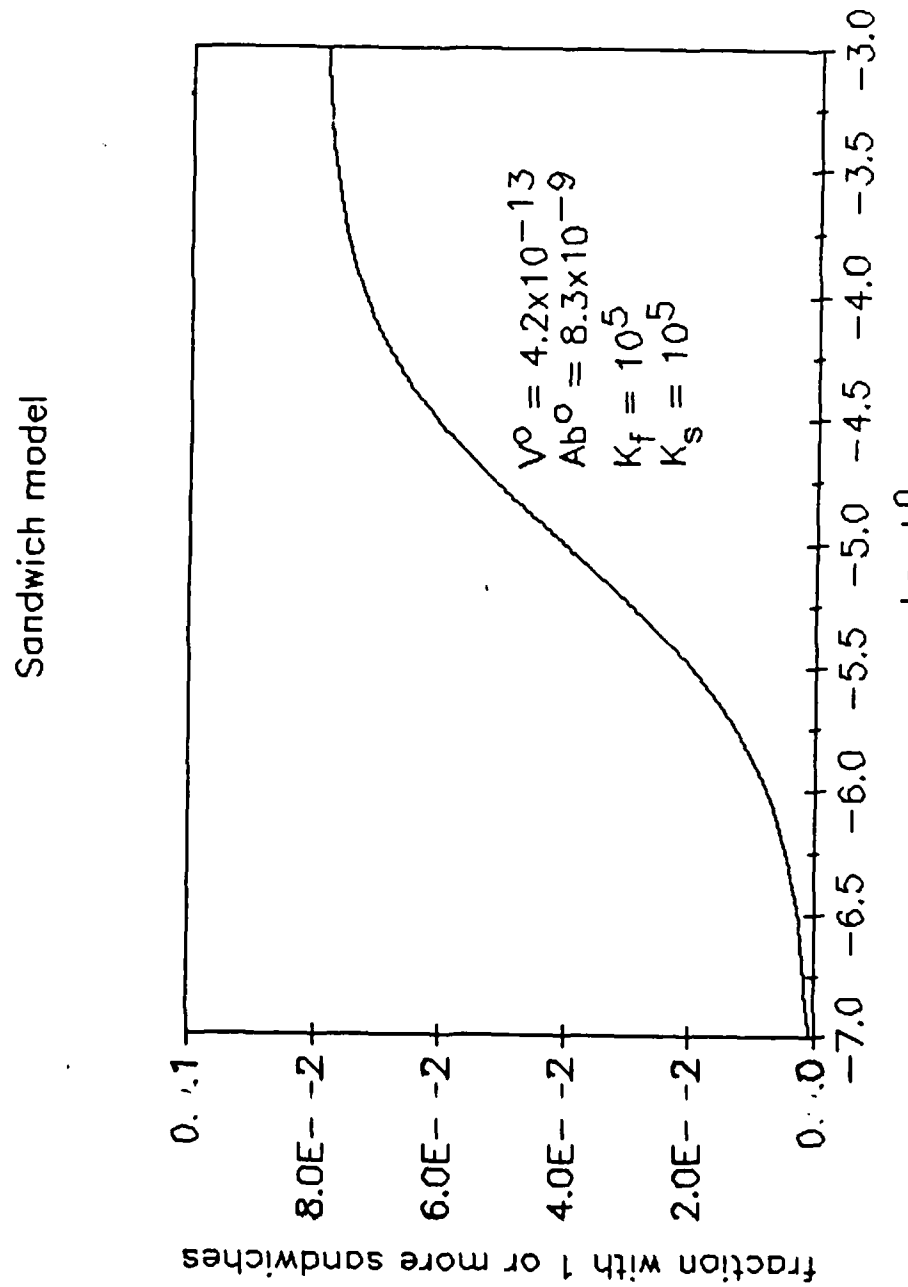

Sandwich model
fraction with 1 or more sandwiches
0.1
8.0E-2
6.0E-2
4.0E-2
2.0E-2
0.0
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
Log L°
V° = 4.2x10⁻¹³
Ab° = 8.3x10⁻⁹
K_f = 10^5
K_s = 10^5

Figure 10

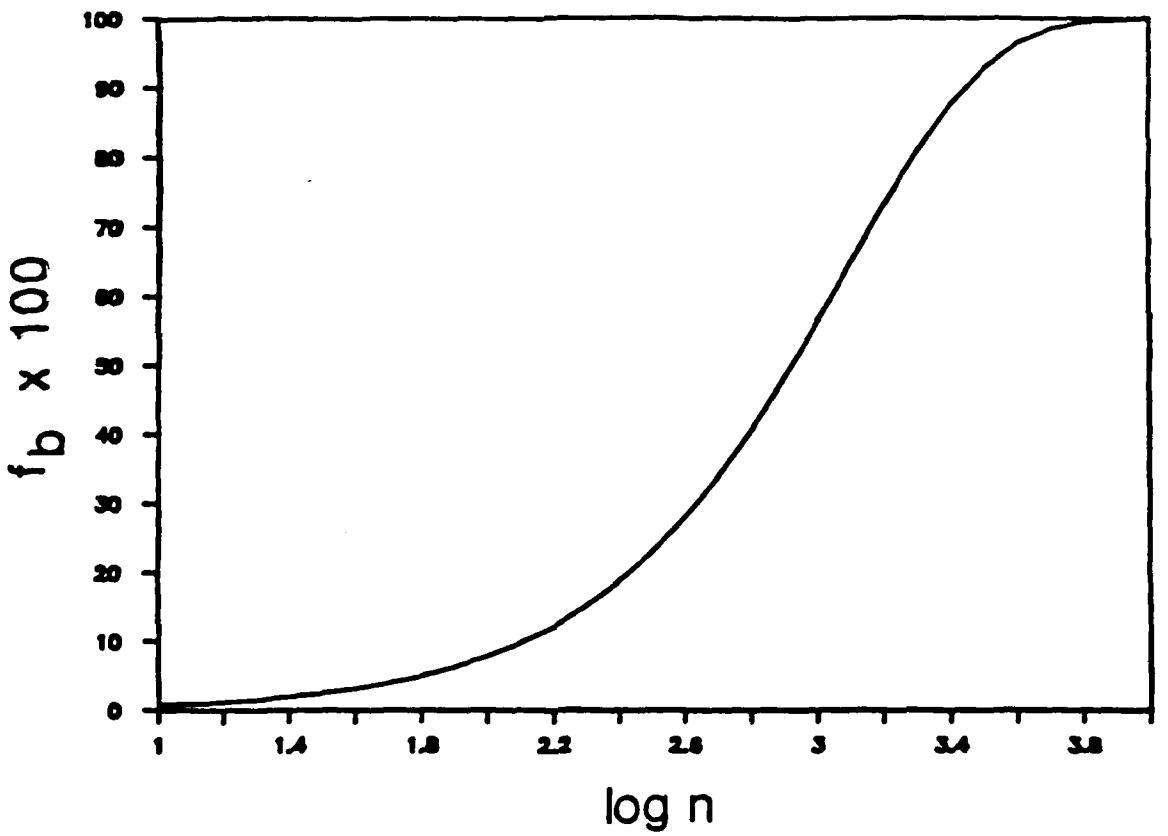


Figure 11 - Monovalent receptor-Monovalent ligand model. $Ab^o = 8.3 \times 10^{-9}$ M. Number of receptors (n) vs. fraction with antibody ligands attached.

Plot from Waite and Chang's mono-mono paper (used by permission)

reach seven percent. This shows that the sandwich model is less sensitive than the direct binding model. This result is to be expected since this model requires a two step process to be completed versus a one step process. The plots also show a smooth increase in the number of sites "sandwiched" up to a maximum. On the plot which varies Ab^o, the maximum is one. This plot will not drop back off, as does the monovalent receptor, bivalent ligand model, shown in Figure 15 [15], since there isn't any competition between the links and the ligands to get to the receptor cites. There is no competition since the links are specific to the receptor on one end and the ligand on the other. The plot of varying L^o concentrations reaches a maximum at approximately eight percent. This is logical since it was shown that the sandwiches form at higher concentrations of Ab^o on the first plot, then the low concentration of the Ab^o in this plot lowers the possible number of sandwiches. The number of links attached does not affect the number of sandwiches after a point if there are not enough ligands present to form the second step of the process.

The values of 10^5 for the binding constants are typical [16] but can vary from system to system. This system is a simple two step process. Changing one binding constant by some orders of magnitude, while holding the second constant, then changing the second constant

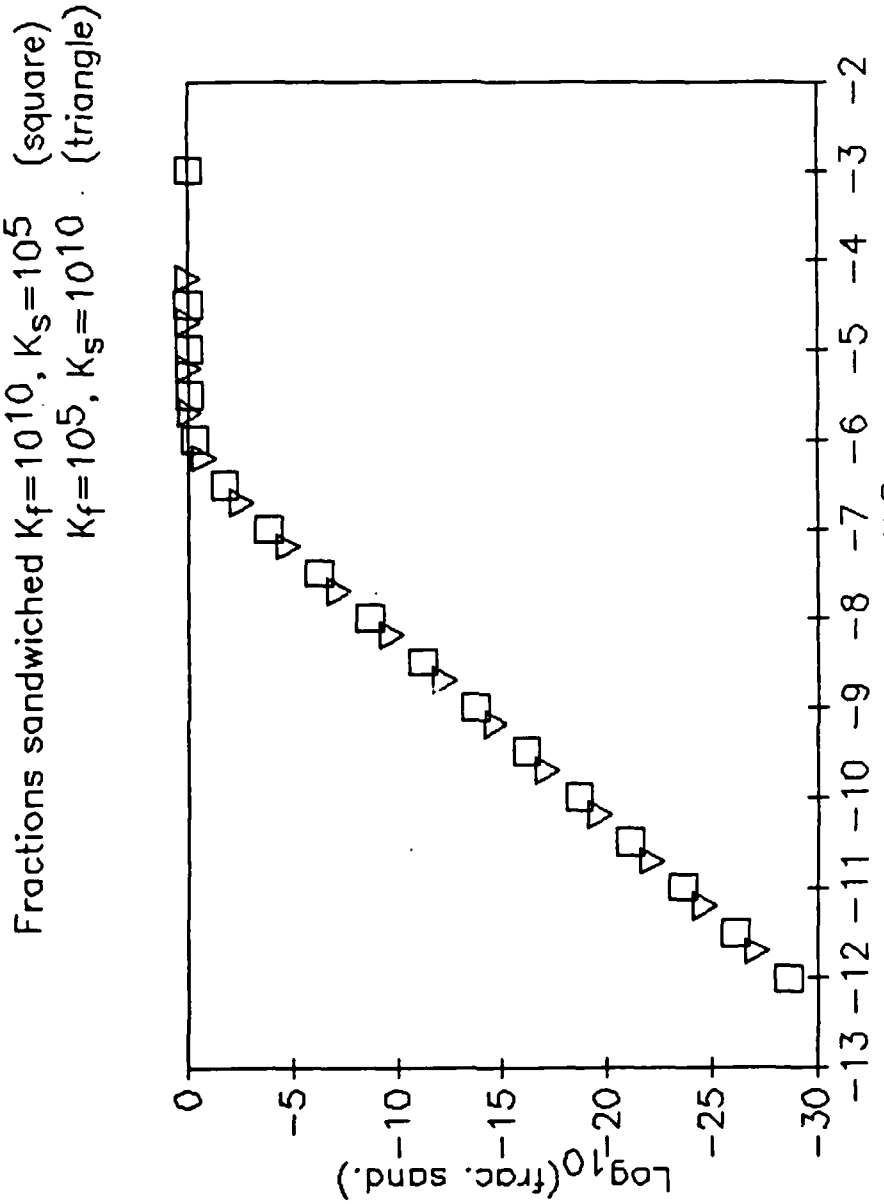

Fractions sandwiched $K_f=10^{10}$, $K_s=10^5$ (square)
$K_f=10^5$, $K_s=10^{10}$ (triangle)
$\log_{10}$(frac. sand.)
0
-5
-10
-15
-20
-25
-30
-13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2
$\log_{10}$ Ab°

Figure 12

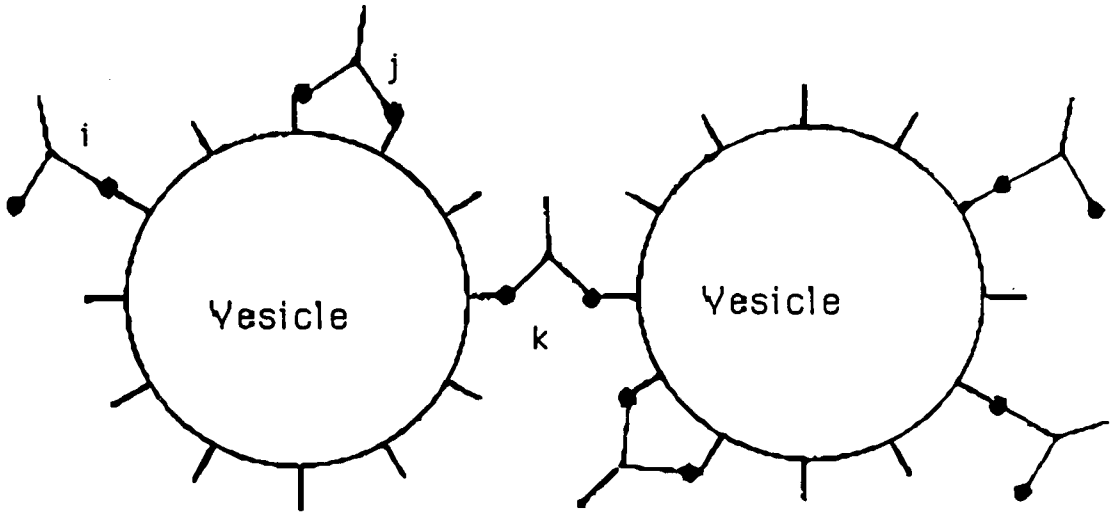
while holding the first to its original value keeps the results constant, as is shown in Figure 12. It is not pictured but is logical that an increase in a single binding constant or increasing both would increase the relative response.

IV. AGGLUTINATION MODEL

Another model which can be used to represent the B-cell response is the monovalent receptor, multivalent antibody, with possibility of inter-linking between vesicles, called agglutination [4]. This is diagrammed in Figure 13. The antibody can be either IgG or IgM. The IgG antibody has two binding sites, while the IgM has ten binding sites, (λ = 2 for IgG, λ = 10 for IgM, where λ is the number of binding sites on the antibody) but for simplicity in this model it will be assumed that only two sites will be available for binding at one time. Due to steric considerations of the planar shape of the IgM, this approximation is not too severe.

From Waite and Chang's paper on multivalency effects in the direct binding model the final equilibrium equation for a single vesicle with monovalent receptors and multivalent ligands is

Agglutination Model
Monovalent receptor-bivalent ligand



a

Figure 13

$$V_{i,j} = d_{i,j}(K_1/\alpha)^j(\lambda K_1 a)^{i+j}V_{o,o} \qquad (23)$$

where

$$d_{i,j} = n!/[i!j!2^j(n-i-2j)!] \qquad (24)$$

and $V_{o,o}$ is the concentration of vesicles without any antibodies bound, either as danglers (i or i′) or two-footers(j or j′), K_1 is the intrinsic association constant, K_2 is the intra-cross-linking constant, and α is the geometric factor which takes into account the local concentration enhancement of forming a two-footer from a dangler.

Now the concentration of the agglutinated vesicles can be derived from this information, with some modifications. For mathematical simplicity, the number of vesicles which can form inter-cellular links will be limited to two cells. Therefore the degeneracy of the agglutinated vesicles can be shown to be the degeneracy of a single vesicle squared with the number of bridges (k) taken into account.

$$d_{agglut} = n!n!/[i!j!2^j(n-i-2j)!k!i'!j'!2^{j'}(n-i'-2j')!] \qquad (25)$$

K_i is the binding constant for the initial bridge, K_z is the binding constant for the bridges following the initial bridge, and β is the geometric factor which takes the steric effects and local concentration effects

for bridging into account. Using the Law of Mass Action as has been shown before, the equilibrium equation for the concentration of two agglutinated vesicles (C_2) can be given by

$$C_2 = d_{agglut}(K_2/\alpha)^j(\lambda K_1 a)^{i+j}V_{o,o}(K_2/\alpha)^{j'}(\lambda K_1 a)^{i'+j'}V_{o,o} K_i(K_2/\beta)^{k-1} \quad (26)$$

which can be simplified to

$$C_2 = d_{agglut}(K_2/\alpha)^{j+j'}(\lambda K_1 a)^{i+i'+j+j'}V^2_{o,o}K_i(K_2/\beta)^{k-1} \quad (27)$$

Equations 23 and 27 can be combined to give the conservation equation, which has been abbreviated.

$$V^o = \sum_{i}^{(n)}\sum_{j} V_{i,j} + 2\sum_{k=1}^{n}\sum_{i}^{(n-f)}\sum_{j}^{(n-f)}\sum_{i'j'} C_2 \quad (28)$$

Combining similar terms and substituting allow the simplified equations to be found.

$$V^o = G(n)V_{o,o} + H(n)V^2_{o,o} \quad (29)$$

where

$$G(n) = \sum_{i=0}^{n/2\downarrow} S(i)(x^i + x^{n-i})/(1+\delta_{n/2,i}) \quad (30)$$

The $n/2\downarrow$ means the term will be truncated, and the Kronecker delta function $\delta_{i,j} = 1$ for $i = j$ and $\delta_{i,j} = 0$ for $i \neq j$.

$$S(i) = \sum_{j=0}^{i} n!y!/[(i-j)!j!2^j(n-i-j)!] \tag{31}$$

and

$$H(n) = \sum_{k=1}^{n} \{n!n!/[k!(n-k)!(n-k)!]\}x^k z^{k-1} K_i 2G(n-k) \tag{32}$$

$$x = aK_1$$

$$y = K_2/\alpha$$

$$z = K_z/\beta$$

The antibody conservation equation can be described in similar terms

$$Ab^o = a + J(n)V_{o,o} + W(n)V^2{}_{o,o} \tag{33}$$

$J(n)$ and $W(n)$ are defined as

$$J(n) = \sum_{i=0}^{n/2\downarrow} (ix^i + (n-i)x^{n-i})S(i)/(1 + \delta_{n/2,i}) \tag{34}$$

$$W(n) = \sum_{k=1}^{n} \{n!n!/k!(n-k)!(n-k)!\}x^k z^{k-1} K_i [kG^2(n-k) + 2G(n-k)J(n-k)] \tag{35}$$

and x, y, and z are defined as before.

AGGLUTINATION MODEL RESULTS

The agglutination model is useful for modeling many systems. It can be used to model the immunolysis assays with monovalent (Fab fragment) receptors and IgG antibody or bivalent antigen ligands or bridging between the AIDS viruses in immunoadhesin experiments [8]. Figure 14 shows the fraction agglutinated of the system with realistic binding constants for the formation of two-footers (j) and danglers (i) while the binding constant for the formation of the initial bridge is varied. This is done since no realistic value for this binding constant is known. The values of K_i on this plot vary from values of 10^{-11} to 10^3. A desired value would be to find the antibody concentration where the agglutination would be maximized. It is not intuitive that the maximum be consistent for all values of K_i, but as the plot shows, no matter what the probability of forming the first bridge is, the maximum occurs at the same concentration, about 5.0×10^{-6} M.

From Waite and Chang's paper on multivalency effects [15] on the direct binding model it was shown that the maximum value for two-footers is about 5.0×10^{-6} M (shown in Figure 15). This maximum is the same for fraction agglutinated, which implies that the two are related. In low antibody concentration very few danglers are formed since the local concentration of receptors is so high that

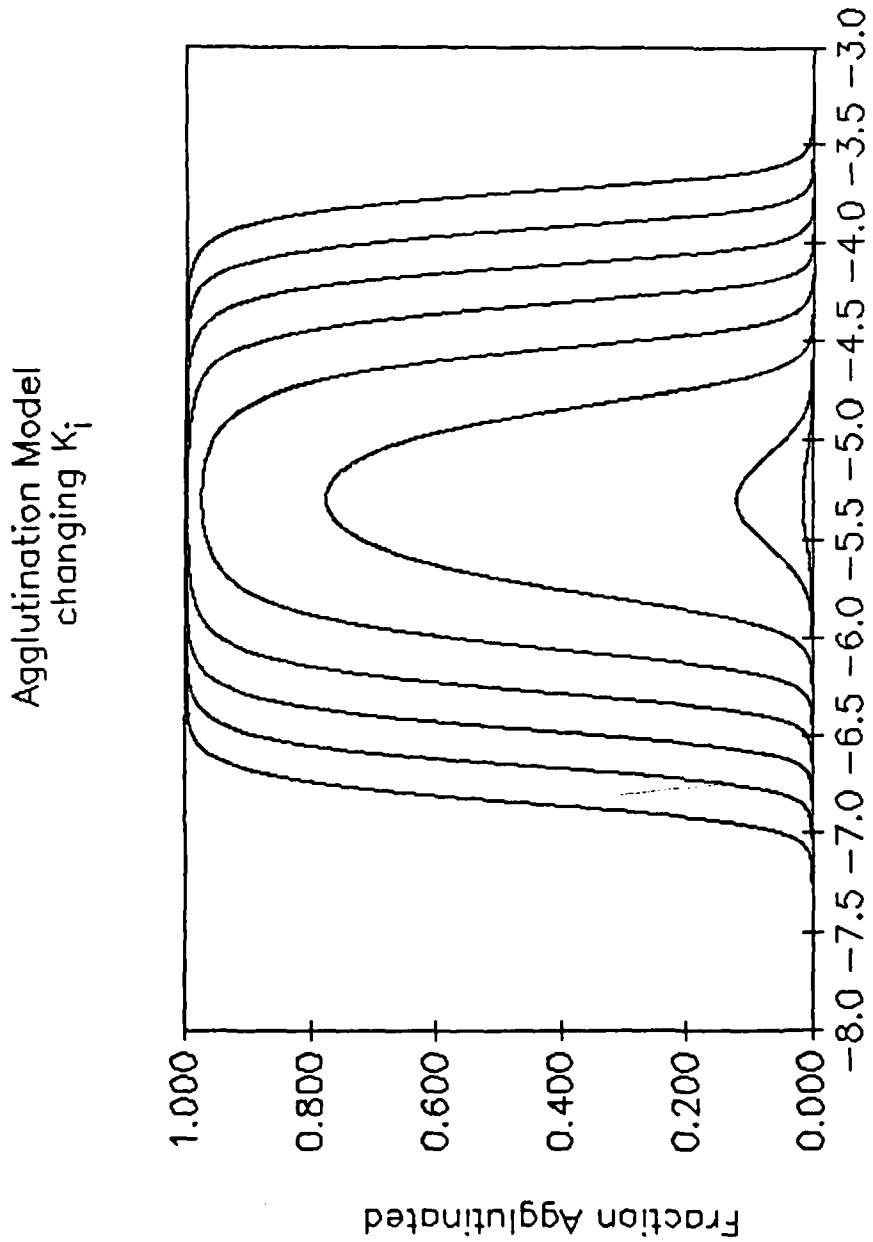

Agglutination Model
changing K_i
Fraction Agglutinated
1.000
0.800
0.600
0.400
0.200
0.000
−8.0 −7.5 −7.0 −6.5 −6.0 −5.5 −5.0 −4.5 −4.0 −3.5 −3.0
Log Ab0

Figure 14

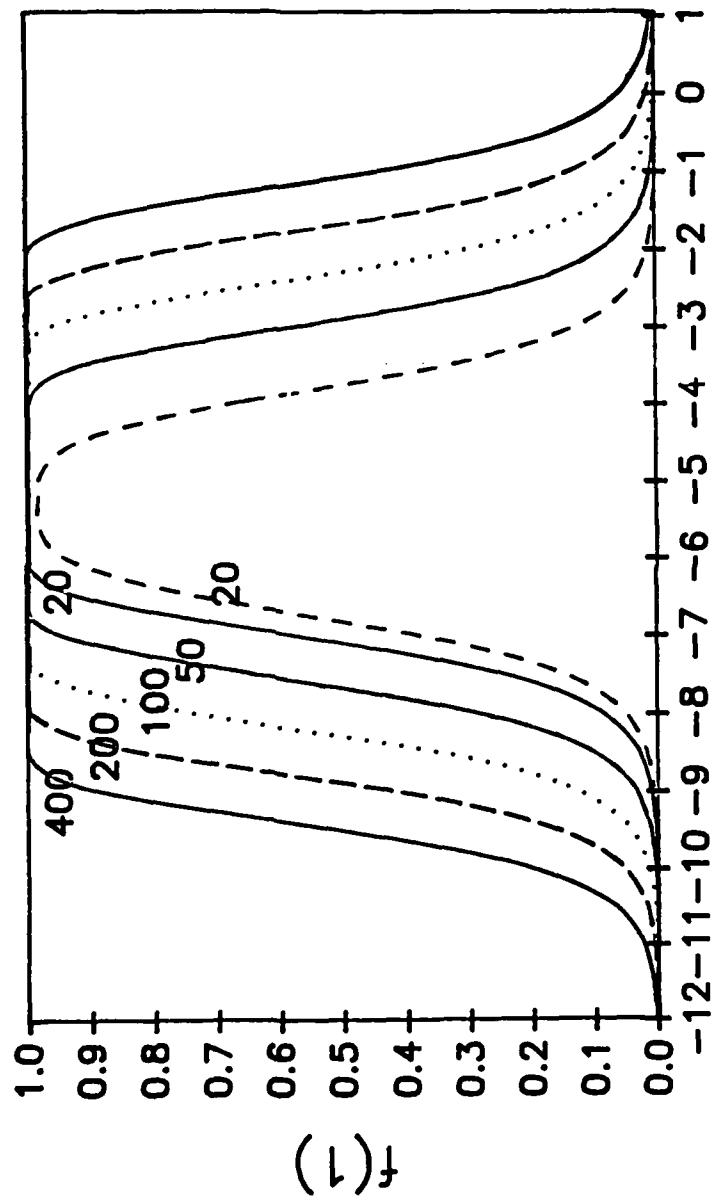


Figure 15 - Plot from Waite and Chang's Multivalency effects paper, used by permission.
Plot of antibody concentration vs. fraction with antibody, two-footer attached.

most move directly to two-footers. A bridge cannot be formed if both sites on the antibody are occupied, therefor it requires danglers to be present. After the maximum concentration of two footers is reached, the relative number of two-footers per vesicle drop due to competition with danglers for receptor sites. These danglers form into bridges. The fraction agglutinated drops off when the antibody concentration gets too high. This is caused by all of the vesicle's receptors being tied up, until finally there are no open receptors on either vesicle, and therefore no bridging.

Figure 16 shows the effects of number of sites per vesicle (n) upon the fraction agglutinated. This curve is relevant since the computer time used increases rapidly with increasing n and realistic numbers of receptor sites would take too long to calculate, if possible. If the effects were to form a smooth curve the effects could be extrapolated to a realistic number of receptors per vesicle.

This plot uses an antibody concentration of 10^{-12} M, to set a low value to try and approximate the threshold detection value for different methods. The light scattering techniques in Cohen and Benedek's papers [17,18] rely on agglutination for detection. Their papers describe laser light and quasi-elastic light scattering from the early stages of agglutination for low level

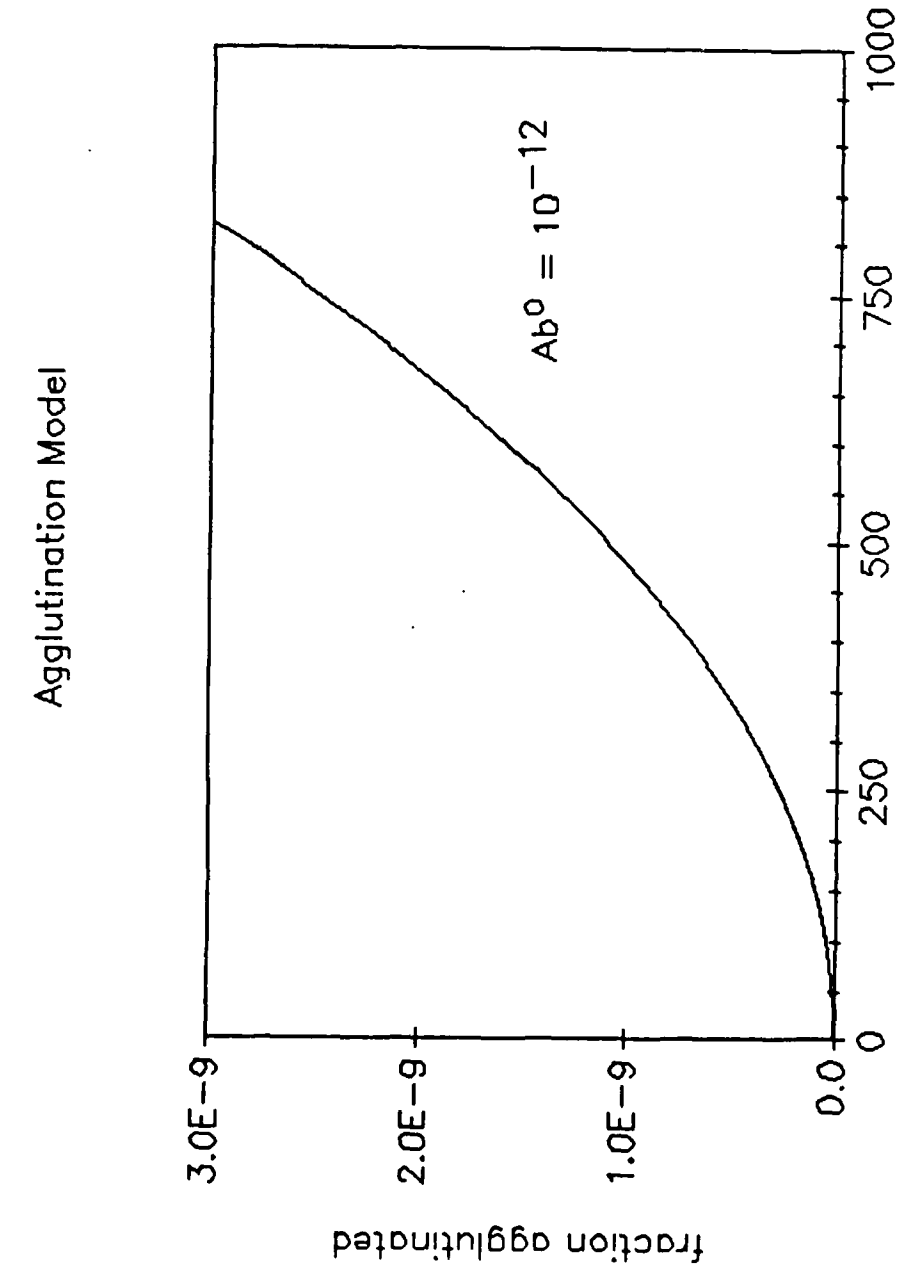

Agglutination Model
fraction agglutinated
3.0E-9
2.0E-9
1.0E-9
0.0
0
250
500
750
1000
n
$Ab^o = 10^{-12}$

Figure 16

detection of ligands. The plot could also be related to the immunolysis experiment which relies on the self-quenching fluorescent compound carboxyfluorescein, and the lowest level of light detectable by the light meter to show a response. This diagram used an arbitrary fraction of agglutination of $2x10^{-9}$, which relates to $8.4x10^{-22}$ M of the vesicles to be lysed, to estimate the detection threshold. At this lower limit of agglutination detection, the vesicles would need at least 675 receptor sites to detect an antibody concentration of 10^{-12} M.

V. NEW DERIVATIONS

Two more models have been derived, but have not been programmed. These models are extensions of the two which have been presented above.

A. Antibody Multivalency Effects on the Sandwich Model

The sandwich model can be made more realistic by taking into account antibody multivalency. As before, the IgG will be represented ideally and the IgM will be limited to an effective binding number of two to limit the mathematical complexity.

This model again uses the vesicle with n receptor sites which can be involved first with the linking ligand, then the antibody. The degeneracy of this system is

$$d_{f,s,t} = n!/[f!s!t!2^t(n-f-s-2t)!] \qquad (36)$$

Figure 17 shows this model where f receptor sites have a link attached, s with an antibody bound to a link singularly, in a similar manner as a dangler, and t with links bound by the same antibody.

The Law of Mass Action equations necessary to derive the equilibrium equations are

$$V'_{f,s,t} \overset{K_t}{=} V'_{f,s+1,t-1} \overset{t \text{ times}}{\Rightarrow\Rightarrow\Rightarrow} V_{f,s+t,o} \qquad (37)$$

$$V'_{f,s+t,o} \overset{K_s}{=} V'_{f+1,s-1,o} + a' \overset{s+t \text{ times}}{\Rightarrow\Rightarrow\Rightarrow} V_{f+s+t,o,o} + a \qquad (38)$$

$$V'_{f+s+t,o,o} \overset{K_f}{=} V'_{f+s+t-1,o,o} + \ell' \overset{f+s+t \text{ times}}{\Rightarrow\Rightarrow\Rightarrow} V_{o,o,o} + \ell \qquad (39)$$

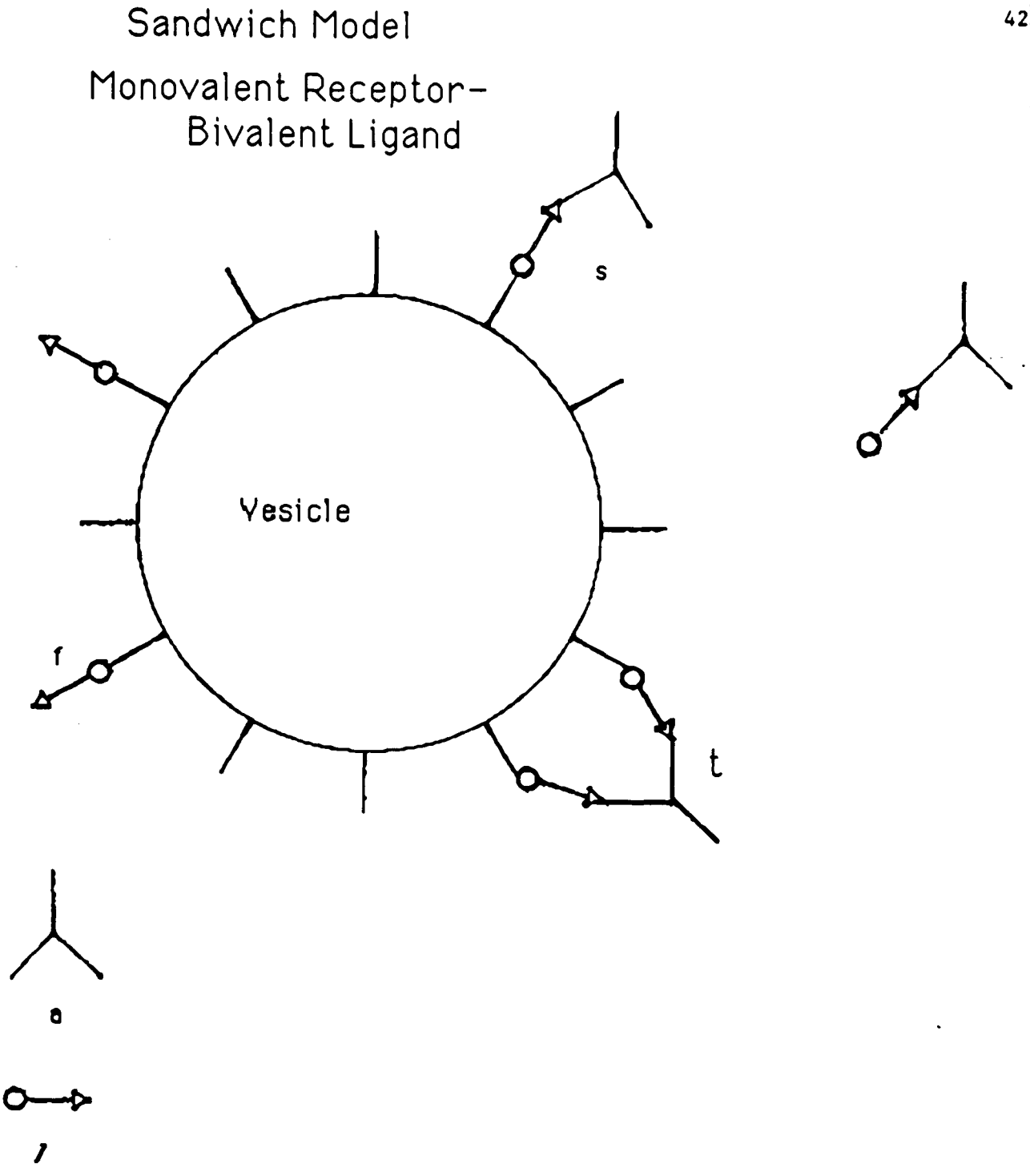

Sandwich Model
Monovalent Receptor-
Bivalent Ligand
s
Vesicle
f
t
a
l

Figure 17

These equations taken to the limits shown above yield the equilibrium equation

$$V_{f,s,t} = d_{f,s,t}(K_t/\alpha)^t(K_s a\lambda)^{s+t}(K_f \ell)^{f+s+t}V_{o,o,o} \tag{40}$$

λ is the number of receptor sites on each antibody and α is the geometric factor which takes local concentration effects into account.

Defining $x = K_f\ell$, $y = K_s a$, and $z = K_t/\alpha$ the following conservation equations can be derived.

$$V^o = \sum_{t=0}^{n-f-s/2} \sum_{s=0}^{n-f} \sum_{f=0}^{n} d_{f,s,t}x^{f+s+t_y}s+t_z t V_{o,o,o} \tag{41}$$

$$Ab^o = \sum_{t=0}^{n-f-s/2} \sum_{s=0}^{n-f} \sum_{f=0}^{n} d_{f,s,t}(s+t)x^{f+s+t_y}s+t_z t V_{o,o,o} \tag{42}$$

$$L^o = \sum_{t=0}^{n-f-s/2} \sum_{s=0}^{n-f} \sum_{f=0}^{n} d_{f,s,t}(f+s+2t)x^{f+s+t_y}s+t_z t V_{o,o,o} \tag{43}$$

Using Equation 9 and its derivative the conservation equations can be simplified into

$$V^{o} = (1+x)^{n}(1+xy)^{n-f}H(t) \tag{44}$$

$$Ab^{o} = a + 2K_{s}a\ell + K^{2}{}_{s}a^{2}\ell + (n-f)xy(1+x)_{n}(1+xy)^{n-f-1}H(t)V_{o,o,o} + (1+x)^{n}(1+xy)^{n-f}tH(t)V_{o,o,o} \tag{45}$$

$$L^{o} = \ell + 2K_{s}a\ell + K^{2}{}_{s}a^{2}\ell + nx(1+x)^{n-1}(1+x)^{n-f}H(t)V_{o,o,o} + (1+x)^{n}(n-f)xy(1+xy)^{n-f-1}H(t)V_{o,o,o} + (1+x)^{n}(1+xy)^{n-f}2tH(t)V_{o,o,o} \tag{46}$$

where

$$H(t) = \sum_{t=0}^{n-f-s/2} (n-f-s)!/[t!2^{t}(n-f-s-2t)!]\ (xyz)^{t} \tag{47}$$

The results from this model would be expected to be related to the monovalent ligand sandwich model much in the same manner as Waite and Chang's papers on monovalent direct binding and multivalency effects are related [14,15]. It would be expected that these effects include geometric factors for local concentration of receptors after one end of the antibody is attached. It is also logical that the number of sandwiches with both sites on

the ligand being attached having a peak when compared to initial antibody concentration. This would be caused by the competition of the "two-footed sandwiches" with the danglers for receptor sites with links attached.

B. Bivalent Receptor/Bivalent Ligand Model

The bivalent receptor, bivalent ligand model is the most complex model presented. The vesicle surface is covered with n surface immunoglobulins (sIg) which each have two receptor cites. This system is shown in Figure 9.

Theoretically a B-cell has antibody chains from surface Ig to sIg which are up to twenty receptors long. These chains can form loops upon themselves, long continuous chains, or large clusters. The number of possible arrangements is mathematically intractable. To make this model's derivation somewhat straightforward this model limits the chaining effects to two sIg. The possible arrangements of two receptor cites is also shown on Figure 9.

The degeneracy of this system need to take this 2^n factor into account, but this falls out since all possible combinations of antibiotic attachment and empties also have this 2^x factor.

Bivalent Receptor-Bivalent Ligand Model

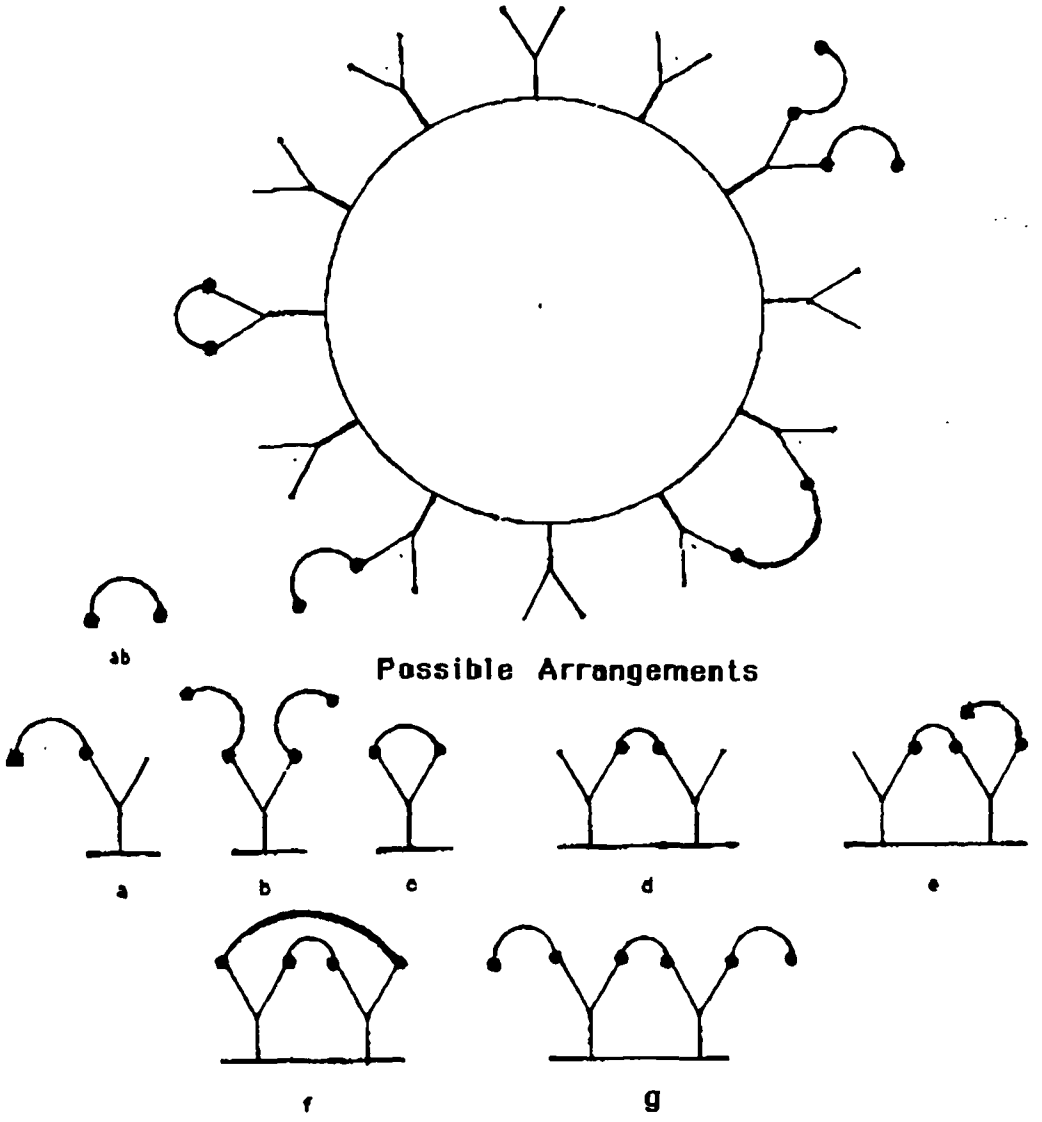


Figure 18

$$d_{a-g} = n!/[a!b!c!d!e!f!g!(n-a-b-c-2d-2e-2f-2g)!] \quad (48)$$

The Law of Mass action equations are shown diagrammatically on Figures 19a and 19b.

The breakdown of each structure is not always a singular path, but this system falls under the same rules as a state function, thus any one pathway may be selected for each combination. The one pathway chosen must be consistent for all the Mass Action equations. The equilibrium equation for any specific combination of antibodies is given by

$$V_{a-g} = d_{a-g}(K_a ab)^{a+b+c+d+2e+2f+2g}(K_b ab)^{b}(K_c)^{c}(K_d)^{d} (K_e)^{e+f+g}(K_f)^{f}(K_g ab)^{g} \quad (49)$$

This equation uses ab to represent free antibody concentration to eliminate confusion between it and receptor-ligand combination a. The binding constants K_b through K_g all have geometric local concentration effects tied into their values.

Bi-Bi Model Law of Mass Action "Equations"

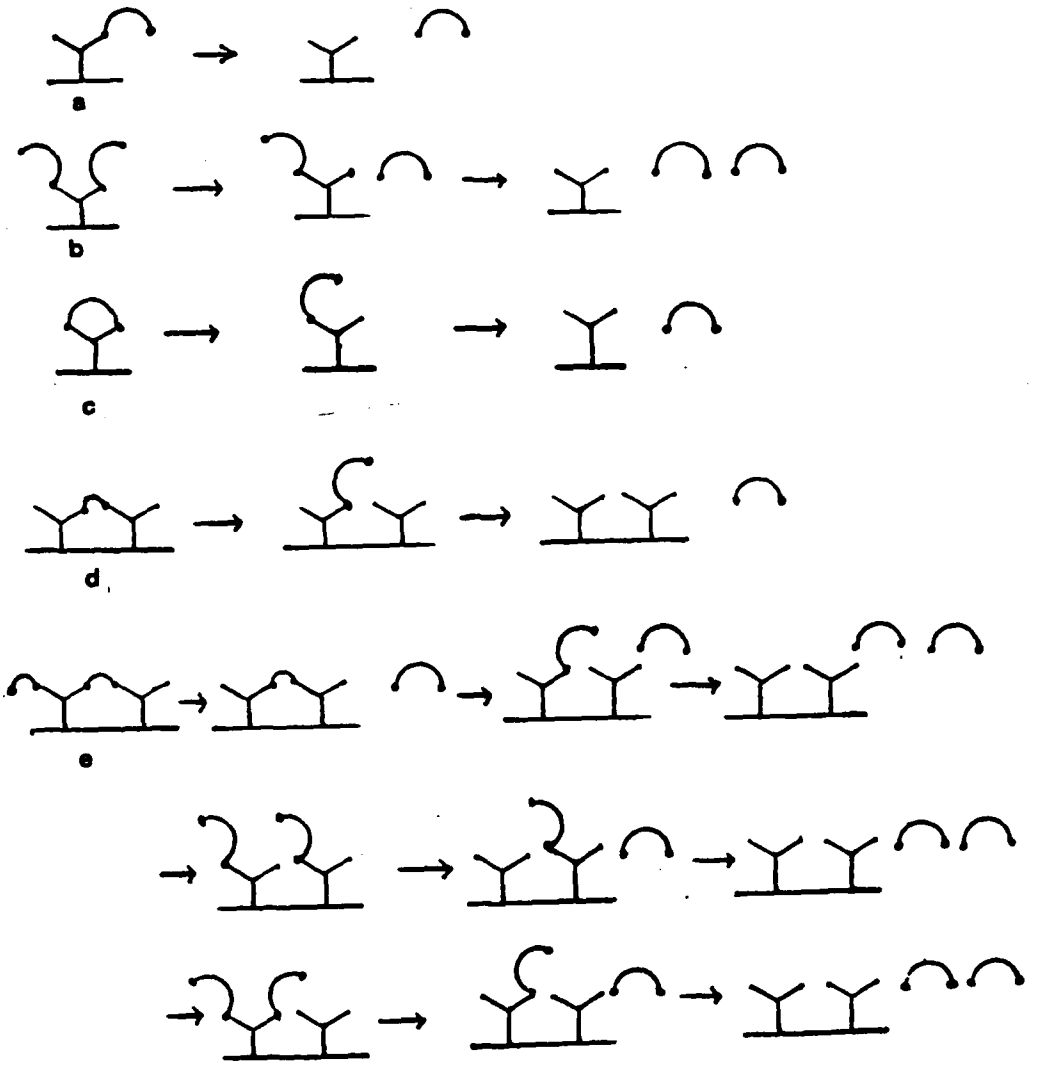
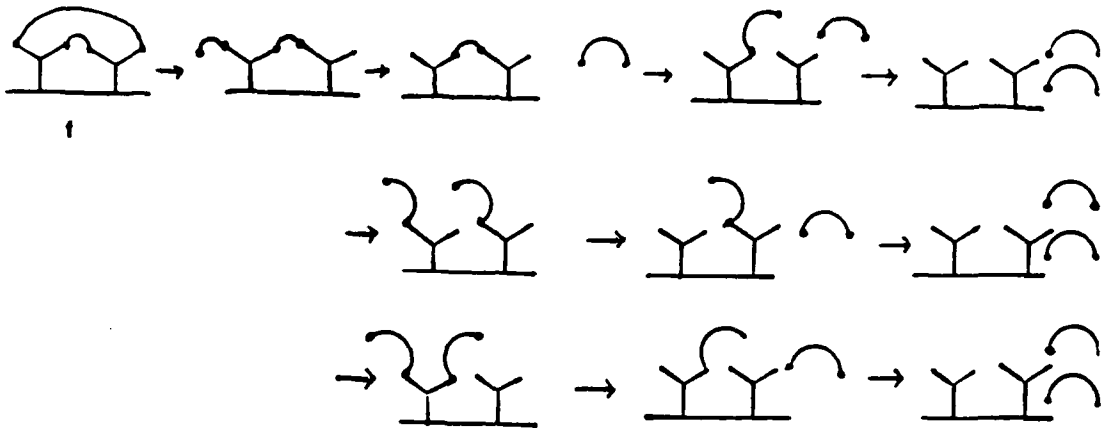
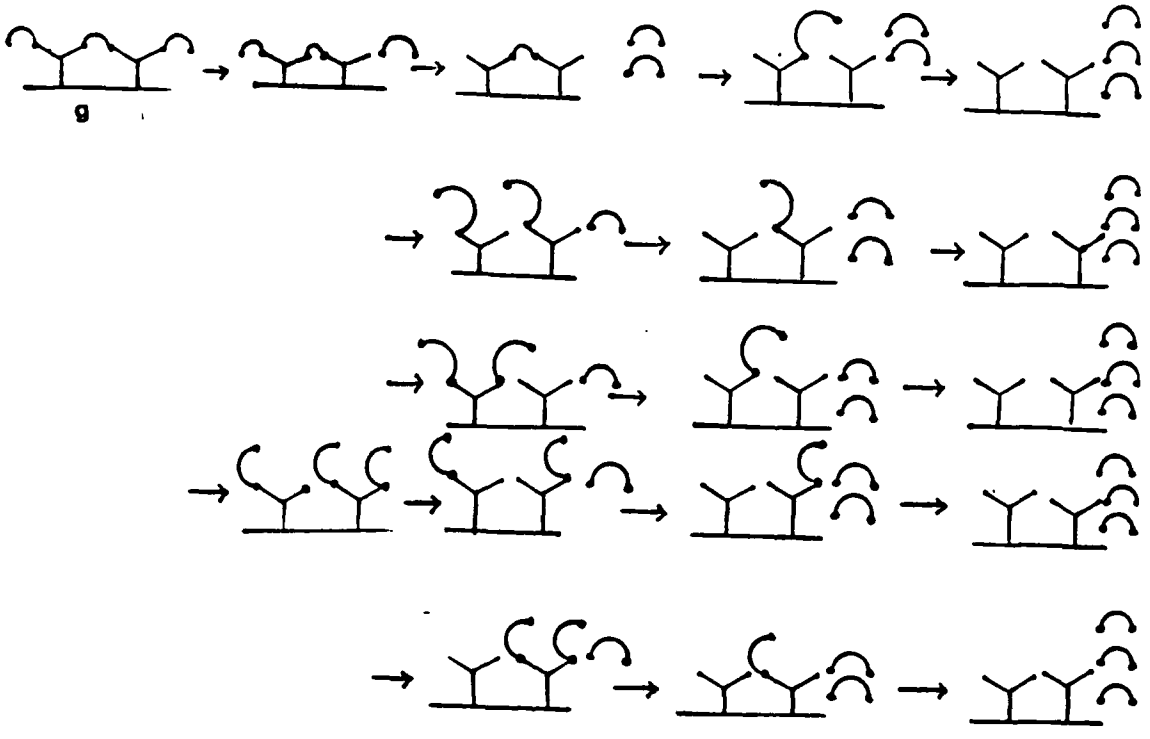


Figure 19a



f



g

Figure 19b

The conservation equation for the vesicles is

$$V^{o} = \sum_{g=0}^{\xi} \sum_{f=0}^{\phi} \sum_{e=0}^{\epsilon} \sum_{d=0}^{\omega} \sum_{c=0}^{\zeta} \sum_{b=0}^{n-a} \sum_{a=0}^{n} d_{a-g} (K_a ab)^a (K_a K_b ab^2)^b$$

$$(K_a K_c ab)^c (K_a K_d ab)^d (K^2_a K_e ab^2)^e (K^2_a K_e K_f ab^2)^f (K^2_a K_e K_g ab^3)^g$$

$$\zeta = n-a-b$$
$$\omega = n-a-b-c/2$$
$$\epsilon = n-a-b-c-2d/2$$
$$\phi = n-a-b-c-2d-2e/2$$
$$\xi = n-a-b-c-2d-2e-2f/2$$

(50)

Models have been derived with similar arrangements [21] of bivalent ligands and bivalent receptors, but again, this model is receptor-carrier based [10]. This will allow questions about linked receptors per vesicle to be answered.

VI. CONCLUSION

The purpose of this paper was to describe in detail the derivation of a theoretical model for antigen-antibody interaction and how that interaction relates to the immune response, immunoassay methods of detection, and the use of immunoadhesins for AIDS therapy. These models were receptor carrier based which allows the questions of number of interactions per vesicle or cell to be addressed. The results may be used to discover parameters for these systems. With help from experimental immunologists to relate real known binding constants and concentrations, boundary limits and interactions on the cell/vesicle surface may be described. These models can be used as building blocks for describing more complex systems.

REFERENCES

1 L.E. Hood, I.L. Weissman, W.B. Wood, and J.H. Wilson, Immunology, The Benjamin/Cummings Publishing Company, Inc., London, 1984.

2 W.T. Keeton, and J.L. Gould, Biological Science, 4th ed., W.W. Norton and Company, New York, 1986.

3 L.A. Segal (Ed.), Mathematical Models in Molecular and Cellular Biology, Cambridge University Press, New York, 1980.

4 E.T. Maggio (Ed.), Enzyme-Immunoassay, CRC Press, Inc., Boca Raton, 1980.

5 G. Gregoriadis, Liposome Technology, CRC Press, Inc., Boca Raton, 1984.

6 R. Wei, C.R. A. Vomg. R.L. Richards, and E.S. Copeland, "Liposome Spin Immunoassay: A New Sensitive Method for Detecting Lipid Substances in Aqueous Media," J. Immunol. Methods 9: 165 (1975).

7 J.N. Weinstein, E. Ralston, L.D. Leserman, R.D.

7 J.N. Weinstein, E. Ralston, L.D. Leserman, R.D. Klausner, P. Dragsten, P. Henkart, and R. Blumenthal, in Liposome Technolgy III, Ed. G. Gregoriadis, CRC Press, Inc., Boca Raton, 1984.

8 D.J. Capon, S.M. Chanow, J. Mordenti, S.A. Marsters, T. Gregory, H. Mitsuya, R.A. Byrn, C. Lucas, F.M. Wurm, J.E. Groopman, S. Broder, and D.H. Smith, "Designing CD4 Immunoadhesins for AIDS Therapy," Nature 337: 525 (1989).

9 C.L. Hornick, and F. Karush, "Antibody Affinity III. The Role of Multivalency," Immunochemistry 9: 325 (1972).

10 B.A. Waite and E.L. Chang, "Models of Immunolysis Assays: A Vesicle-Based Approach for Direct Binding," in Theoretical Immunology, Part Two, Ed. A.S. Perelson, Addison Wesley Publishing Company, Inc., New York, 1988.

11 A.S. Perelson, "Some Mathematical Models of Receptor Clustering by Multivalent Ligands," Cell Surface Dynamics, Eds. A.S. Perelson, C. DeLisi, and F.W. Wiegel, Marcel Dekker, Inc., New York, 1984.

12 V. Brendel, and A.S. Perelson, "Kinetic Analysis of Adsorption Processes," SIAM J. Appl. Math., in press, 1987.

13 S.J. Singer and G.L. Nicolson, "The Fluid Mosaic Model of the Structure of Cell Membranes," Science 175: 720 (1972).

14 E.L. Chang and B.A. Waite, "Analysis of Vesicle Immunolysis Assays: The Direct Binding Model," J. Immunol. Methods 102: 33 (1987).

15 E.L. Chang and B.A. Waite, "Antibody Multivalency Effects in the Direct Binding Model for Vesicle Immunolysis Assays," J. Immunol. Methods 115: 227 (1988).

16 J.A. Benxofsky and I.J. Berkower, "Antigen-Antibody Interaction," in Fundamental Immunology, Ed. W.E. Paul, Raven Press, New York, 1984.

17 R.J. Cohen and G.B. Benedek, "Immunoassay by Light Scatter Spectroscopy," Immunochemistry 12: 249 (1975).

18 R.J. Cohen, G.B. Benedek, N. Sakato, G.K. Von Schulthess, "Laser Light Scattering Spectroscopic Immunoassay for Mouse IgA," Immunochemistry 13: 955 (1976).

19 J.F. LePage, private communication.

20 H.M. Dintzis and R.Z. Dintzis, "A Molecular Basis for Immune Regulation: The Immunon Hypothesis," in Theoretical Immunology, Part One, Ed. A.S. Perelson, Addison-Wesley Publishing Company, Inc., New York, 1988.

21 S.K. Dower, J.A. Titus, and P.M. Segal, "The Binding of Multivalent Ligand to Cell Surface Receptors," in Cell Surface Dynamics, Eds. A.S. Perelson, C. DeLisi, and F.W. Wiegel, Marcel Dekker, Inc., New York, 1984.

22 M. Abramowitz and I.A. Stegun (Eds.), Handbook of Mathematical Functions, Dover Publications, Inc., New York, 1964.

APPENDIX 1

Computer programming of these equations does pose some interesting problems. The precision for the VAX is normally e^{-88} and double precision is e^{-300}. One of the problems occurs when adding a series of numbers of greatly different orders of magnitude. Even when working in natural logarithims, the numbers must be changed back to their non logarithmic form to add them. This problem can be avoided by using a series of steps to make the numbers more manageble.

Desired quantity $\ln (x_1 + x_2 + x_3)$

(a) Find: $\ln x_1$, $\ln x_2$, $\ln x_3$

(b) Find largest quantity from (a)

(c) Subtract (b) from all values in (a)

(d) Take the exponential of values in (c)

(e) Sum numbers in d (magnitudes close)

(f) Take ln (e)

(g) Add ln (f) + ln (b)

(h) Equals $\ln (x_1 + x_2 + x_3)$

i.e. Desired $10^4 + 10^{-1}$ (Log_{10} will be used)

(a) $\log 10^4 = 4$, $\log 10^{-1} = -1$

(b) largest = 4

(c) 0,-5

(d) $10^0 = 1$, $10^{-5} = .00001$

(e) 1.00001

(f) log 1.00001 = 4.3×10^{-6}

(g) $4 + 4.3 \times 10^{-6} = 4.0000043$

(h) $10^{(g)} = 10000.1$ as expected

The other complication arises when the factorial for large numbers is needed. This arises in all the degeneracy calculations since n! is a very large number. This can be approximated using Stirling's approximation which says:

$$\ln(n!) = (n+1-1/2)\ln(n+1) - (n+1) + 1/2\ln(2\pi) + 1/12(n+1) - 1/360(n+1)^3 + 1/1260(n+1)^5 \ldots$$

This approximation gives very exact answers without overloading the computer. Most numbers would overflow normal precision since 100! = 9.3×10^{157}, and many would averflow double precision, therefore Stirling's approximation is used whenever n is greater than seven.

Appendix 2

This computer program is used to develop the plots and results for the sandwich model. The loops fill a two-dimensional array for all $V_{f,s}$ combinations. This array is forced into a one-dimensional array where the summation of the fraction can be found. The data is then passed to data files to be interpreted.

```
ccc   PROGRAM:  SAND.FOR
c     To run type:
c     fortran/g_floating sand.for
c     link sand
c     run sand
c
      implicit real*8 (a-h,o-z)
      dimension Vfs(200,200)
      dimension temp(40000)
      dimension temp2(40000)
      akf=1.d5
      aks=1.d5
      ictr=1
      nsand=1
      sum=0
      Vo=dlog(4.2d-13)
      do 5 n=100,101,10
      do 10 ih=900,901,2
      a=8.3*10.**(-ih/100.)
      do 15 im=900,300,-50
      al=10.**(-im/100.)
      x = aks*a
      y = akf*al
      w = aks*a*al
      Voo = Vo-n*dlog(1+x*y+y)
      do 20 if = 0,n
      do 25 is = 0,n-if
      Dfs=fact(n)-fact(if)-fact(is)-fact(n-is-if)
      Vfs(if+1,is+1)=Dfs+(if+is)*dlog(y)+is*dlog(x)+Voo
   25 continue
   20 continue
      nn=n-1
      yy=1+x*y
      aLo=Voo+dlog(n*y)+nn*dlog(yy)
```

```
      a1Lo=Voo+dlog(n*x*y)+nn*dlog(y+yy)
      a2Lo=dexp(aLo)+a1+w+dexp(a1Lo)
      goto 11
      Abo=dlog(n*x*y)+nn*dlog(y+yy)+dlog(1-y/yy)+Voo
      Abo2=a+w+dexp(Abo)
   11 continue
      ictr=1
      do 100 ia=0,n
      do 95 ib=0,n-ia
      if (ib.lt.nsand) goto 95
      Temp(ictr)=Vfs(ia+1,ib+1)
      ictr=ictr+1
   95 continue
  100 continue
      do 110 ic=1,(ictr-1)
      big=temp(1)
      if (temp(ic).gt.big) big=temp(ic)
  110 continue
      sum=0
      do 120 id=1,(ictr-1)
      temp2(id) = temp(id)-big
      temp2(id)=dexp(temp2(id))
      sum=temp2(id)+sum
  120 continue
      sum=sum*dexp(big)
      write(25,999) n
  999 format(' ',' current n value is: ',i5)
      write(25,1000) Abo2
 1000 format(' ',' computed value of Abo is: ',d16.8)
      write(25,1001) a2Lo
 1001 format(' ',' computed value of Lo is: ',d16.9)
      fraction=sum/dexp(vo)
      write(25,1002) nsand,fraction
 1002 format(' ',' fraction with
```

```
     1',i5,'or more sandwiches is: ',d16.8)
      write(20,1003) dlog10(a2Lo),fraction,dlog10(fraction)
      write(21,1004) n,fraction,dlog10(fraction)
1003  format(3e16.8)
1004  format(i5,2e16.8)
  15  continue
  10  continue
   5  continue
      stop
      end
      real*8 function fact(n)
      implicit real*8 (a-h,o-z)
      t1=.5*dlog(2.d0*3.14159265358979d0)
      if (n.gt.6) goto 30
      if (n.eq.0) fact=0.0
      if (n.eq.1) fact=0.0
      if (n.eq.2) fact=dlog(2.d0)
      if (n.eq.3) fact=dlog(6.d0)
      if (n.eq.4) fact=dlog(24.d0)
      if (n.eq.5) fact=dlog(120.d0)
      if (n.eq.6) fact=dlog(720.d0)
      return
  30  z=n+1
      fact=(z-.5)*dlog(z)-z+t1+1./(12.*z)-1./(360.
     1*z**3)+1./(1260.*
     1z**5)-1./(1680.*z**7)
      return
      end
```

Appendix 3

This program is more complex and layered to solve the agglutination model. The functions S(i), G(n) and others which are used more than once have their own arrays to make the calculations easier.

```
ccc   PROGRAM:  BEST.FOR
c        to run, type
c        fortran/g_floating [waite.inter]best.for
c        link best
c        run best
c        type for000.dat
c
c
c     Philosophy of program:
c          sums of large numbers are handled with the
c            logarithmic, reduce by biggest, exp, sum,
c            log, increase by biggest   method.
c          the quadratic formula is implemented with similar
c            consideration given to large/small numbers in
c            function f
c          Sterling's formula is implemented in function fact
c
c
c     The summary of variables possessing the various functions is as
c       follows:
c                                      (nsize)
c           s(nsize+1,k+1)  is the s(i)
c           g(nsize+1) is the g(nsize)
c           j(nsize+1) is the j(nsize)
c           hn  is the h(n)
c           wn  is the w(n)
c           pn  is involved with the m or more bridges calculation
c
c
c
      implicit real*8 (a-h,o-z)
      dimension t1(300),bigest(300),sum1(300),s(300,300)
      dimension zz(300,300),zzn(300,300),sum(300),tut(300)
      dimension uu(300,300),uun(300,300),sum1(300),s1(300)
```

```
      dimension s2(300),s3(300)
      zero=0.0
      mbrdg=3
      do 987 qw=-5,7,1
      ak1=10.**(qw)
      ak2=1.d5
      aki=1.d3
      akz=1.d5
      val=2.0
      alpha=7.d5
      beta=7.d5
      y=ak2/alpha
      z=akz/beta
      vo=4.2d-13
      write(0,2323)
 2323 format(' ',' k1       k2     ki          kz      val')
      write(0,1212) ak1,ak2,aki,akz,val
 1212 format(' ',5d16.8)
      write(0,3434)
 3434 format(' ',' alpha    beta     vo')
      write(0,1212) alpha, beta,vo
      do 7860 n=100,101,10
      write(0,8642)
 8642 format(' ')
      write(0,4545) n
 4545 format(' ','current value for n  is : ',i6)
      do 5 nsize=0,n
      do 15 k=0,nsize/2
      do 25 j=0,k
      t1(j+1)=fact(nsize)-fact(k-j)
     1-fact(j)-fact(nsize-k-j)+j*dlog(y/2.)
   25 continue
      bigest(k+1)=t1(1)
      do 35 j=0,k
```

```
      if(t1(j+1).gt.bigest(k+1)) bigest(k+1)=t1(j+1)
   35 continue
      sum1(k+1)=0.0
      do 45 j=0,k
      t1(j+1)=t1(j+1)-bigest(k+1)
      if(t1(j+1).lt.-300.) goto 102
      t1(j+1)=dexp(t1(j+1))
      goto 101
  102 t1(j+1)=0.0
  101 sum1(k+1)=sum1(k+1)+t1(j+1)
   45 continue
      s(nsize+1,k+1)=dlog(sum1(k+1))+bigest(k+1)
   15 continue
    5 continue
      do 666 i=800,300,-5
      a=10.**(-i/100.)
      write(0,8642)
      write(0,5676) a
 5676 format(' ','  value for free antibody a is :',d16.8)
      x=a*val*ak1
      do 135 nsize=0,n
      do 145 k=0,nsize/2
      t11=k*dlog(x)
      t2=(nsize-k)*dlog(x)
      if(k.eq.0) t3=-10000.
      if(k.eq.0) goto 17
      ak=k
      t3=t11+dlog(ak)
   17 if(nsize.eq.k) t4=-10000.
      if(nsize.eq.k) goto 18
      anmk=nsize-k
      t4=t2+dlog(anmk)
   18 if((nsize/2)*2.eq.nsize) goto 3
      goto 333
```

```
  3 if(k.eq.(nsize/2)) t11=t11-dlog(2.d0)
    if(k.eq.(nsize/2)) t2=t2-dlog(2.d0)
    if(k.eq.(nsize/2)) t3=t3-dlog(2.d0)
    if(k.eq.(nsize/2)) t4=t4-dlog(2.d0)
333 zz(nsize+1,k+1)=s(nsize+1,k+1)+t11
    zzn(nsize+1,k+1)=s(nsize+1,k+1)+t2
    uu(nsize+1,k+1)=s(nsize+1,k+1)+t3
    uun(nsize+1,k+1)=s(nsize+1,k+1) + t4
145 continue
    big=zz(nsize+1,1)
    bigu=uu(nsize+1,1)
    do 56 k=0,nsize/2
    if(zz(nsize+1,k+1).gt.big) big=zz(nsize+1,k+1)
    if(zzn(nsize+1,k+1).gt.big) big=zzn(nsize+1,k+1)
    if(uu(nsize+1,k+1).gt.bigu) bigu=uu(nsize+1,k+1)
    if(uun(nsize+1,k+1).gt.bigu) bigu=uun(nsize+1,k+1)
 56 continue
    do 67 k=0,nsize/2
    zz(nsize+1,k+1)=zz(nsize+1,k+1)-big
    zzn(nsize+1,k+1)=zzn(nsize+1,k+1)-big
    uu(nsize+1,k+1)=uu(nsize+1,k+1)-bigu
    uun(nsize+1,k+1)=uun(nsize+1,k+1)-bigu
 67 continue
    sum(nsize+1)=0.0
    sumu(nsize+1)=0.0
    do 78 k=0,nsize/2
    if(zz(nsize+1,k+1).lt.-300.) goto 578
    zz(nsize+1,k+1)=dexp(zz(nsize+1,k+1))
    goto 478
578 zz(nsize+1,k+1)=0.0
478 continue
    if(zzn(nsize+1,k+1).lt.-300.) goto 579
    zzn(nsize+1,k+1)=dexp(zzn(nsize+1,k+1))
    goto 479
```

```
479 continue
    if(uu(nsize+1,k+1).lt.-300.) goto 679
    uu(nsize+1,k+1)=dexp(uu(nsize+1,k+1))
    goto 680
679 uu(nsize+1,k+1)=0.0
680 continue
    if(uun(nsize+1,k+1).lt.-300.) goto 779
    uun(nsize+1,k+1)=dexp(uun(nsize+1,k+1))
    goto 780
779 uun(nsize+1,k+1)=0.0
780 continue
    sum(nsize+1)=sum(nsize+1)+zz(nsize+1,k+1)
   1+zzn(nsize+1,k+1)
    sumu(nsize+1)=sumu(nsize+1)+uu(nsize+1,k+1)
   1+uun(nsize+1,k+1)
 78 continue
    sum(nsize+1)=dlog(sum(nsize+1))+big
    sumu(nsize+1)=dlog(sumu(nsize+1))+bigu
135 continue
    do 601 k=1,n
    s1(k)=2.*fact(n)+k*dlog(x)+(k-1)*dlog(z)+dlog(aki)-fact(k)-
   1 2.*fact(n-k)
    ak=k
    s2(k)=s1(k)+dlog(ak)+2.*sum(n-k+1)
    s3(k)=s1(k)+dlog(2.d0)+sum(n-k+1)+sumu(n-k+1)
    s1(k)=s1(k)+dlog(2.d0)+2.*sum(n-k+1)
601 continue
    b1=s1(1)
    b2=s2(1)
    do 602 k=1,n
    if(s1(k).gt.b1) b1=s1(k)
    if(s2(k).gt.b2) b2=s2(k)
    if(s3(k).gt.b2) b2=s3(k)
602 continue
```

```
      s1(k)=s1(k)-b1
      s2(k)=s2(k)-b2
      s3(k)=s3(k)-b2
  603 continue
      hn=0.0d0
      wn=0.0d0
      pn=0.0d0
      do 604 k=1,n
      if(s1(k).lt.-300.)goto 720
      s1(k)=dexp(s1(k))
      goto 721
  720  s1(k)=0.0
  721 continue
      if(s2(k).lt.-300.)goto 722
      s2(k)=dexp(s2(k))
      goto 723
  722 s2(k)=0.0
  723 continue
      if(s3(k).lt.-300.) goto 724
      s3(k)=dexp(s3(k))
      goto 725
  724 s3(k)=0.0
  725 continue
      hn=hn+s1(k)
      wn=wn+s2(k)+s3(k)
      if(k.lt.mbrdg) pn=pn+s1(k)
  604 continue
      pn=hn-pn
      hn=dlog(hn)+b1
      wn=dlog(wn) + b2
      pn=dlog(pn)+b1
      bbb=sum(n+1)
      ccc=dlog(vo)
      aaa=hn
```

```
      bog=aaa
      if(bbb.gt.bog)bog=bbb
      if(ccc.gt.bog)bog=ccc
      aaaa=aaa-bog
      bbbb=bbb-bog
      cccc=ccc-bog
      v00=f(aaaa,bbbb,cccc)
 8889 if(v00.gt.-300.)write(0,1111) dexp(v00)
      if(v00.le.-300.)write(0,1111) zero
      term1=dlog(a)
      term2=sumu(n+1)+v00
      term3=wn+2.*v00
      big=term1
      if(term2.gt.big) big=term2
      if(term3.gt.big) big=term3
      term1=term1-big
      term2=term2-big
      term3=term3-big
      stum=0.0
      if(term1.lt.-300.0)goto 851
      term1=dexp(term1)
      goto 852
  851 term1=0.0
  852 continue
      if(term2.lt.-300.) goto 853
      term2=dexp(term2)
      goto 854
  853 term2=0.0
  854 continue
      if(term3.lt.-300.) goto 855
      term3=dexp(term3)
      goto 856
  855 term3=0.0
  856 continue
```

```
 856 continue
     abo=term1+term2+term3
     abo=dlog(abo)+big
     if(abo.gt.-300.) write(0,1113) dexp(abo)
     if(abo.le.-300.) write(0,1113) zero
     write(0,1114) dlog10(dexp(abo))
     guppy=hn+2.*v00-ccc
     if(guppy.gt.-300.) write(0,222) dexp(guppy)
     if(guppy.le.-300.) write(0,222) zero
     patch=pn+2.*v00-ccc
     if(patch.gt.-300.) write(0,242) mbrdg,dexp(patch)
     if(patch.le.-300.) write(0,242) mbrdg,zero
     write(1,111) dlog10(dexp(abo)),dexp(guppy)
     write(2,111) dexp(abo),dexp(guppy)
     write(3,111) dlog10(dexp(abo)),dexp(patch)
     write(4,1121) n,dexp(guppy)
 242 format(' ','    fract. with ',i5,' or more
    1bridges : ',d16.8)
 111 format(' ',3e16.8)
1121 format(' ',i5,3e16.8)
1111 format(' ','    computed value of v00 is: ',d16.8)
1113 format(' ','    computed value of abo is: ',d16.8)
1114 format(' ','    computed value of log(abo) is: ',d16.8)
 222 format(' ','    f(agglut)=  ',d16.8)
 666 continue
7860 continue
 987 continue
     stop
     end
     real*8 function fact(n)
     implicit real*8 (a-h,o-z)
     t1=.5*dlog(2.d0*3.14159265358979d0)
     if(n.gt.6) goto 10
     if(n.eq.0) fact=0.0
```

```
      if(n.eq.1) fact=0.0
      if(n.eq.2) fact=dlog(2.d0)
      if(n.eq.3) fact=dlog(6.d0)
      if(n.eq.4) fact=dlog(24.d0)
      if(n.eq.5) fact=dlog(120.d0)
      if(n.eq.6) fact=dlog(720.d0)
      return
   10 z=n+1
      fact=(z-.5)*dlog(z)-z+t1+1./(12.*z)-1./
     1(360.*z*z*z)+1./(1260.*
     1z*z*z*z*z) -1./(1680.*z*z*z*z*z*z*z)
      return
      end
      real*8 function f(a,b,c)
      implicit real*8 (a-h,o-z)
      x=dlog(4.d0)+a+c-2.*b
  111 format(' ',3d16.8)
      if(x.gt.0.0) goto 15
      if(x.lt.-5.0)goto 5
      x=dexp(x)+1.
      x=dsqrt(x)*dexp(b)-dexp(b)
      f=dlog(x)-dlog(2.d0)-a
      return
    5 t1=dexp(a+c-b-b)
      f=c-b-t1+2.*t1*t1-5.*t1*t1*t1+14.*t1**4-42.*t1**5
      return
   15 y=2*b-a-c
      if(y.lt.-300.)goto 35
      y=dexp(y)
      goto 36
   35 y=0.0
   36 continue
      z=(-dsqrt(y)+dsqrt(y+4.d0))/2.
      f=dlog(z)+.5*c-.5*a
```